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Interactions between a Gall Making Fly, Dasineura Oxycoccana (Diptera: Cecidomyiidae), And Its Host Plant, Cultivated Cranberry (Vaccinium Macrocarpon)

Sunil Tewari
University of Massachusetts Amherst, tewari.sunil@gmail.com

INTERACTIONS BETWEEN A GALL MAKING FLY, *DASINEURA OXYCOCCANA* (DIPTERA: CECIDOMYIIDAE), AND ITS HOST PLANT, CULTIVATED CRANBERRY (*VACCINIUM MACROCARPON*)

A Dissertation Presented

by

SUNIL TEWARI

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Approved as to style and content by:

_____
Anne L. Averill, Chair

_____
Lynn S. Adler, Member

_____
J. P. Buonaccorsi, Member

_____
Daniel R. Cooley, Member

_____
Geunhwa Jung, Member

_____
Curtice R. Griffin, Department Head,
Department of Environmental Conservation.

DEDICATION

To my Mom and Dad.

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I want to thank the cranberry growers in Maine and Massachusetts for allowing me to work on their properties.

ABSTRACT

INTERACTIONS BETWEEN A GALL MAKING FLY, *DASINEURA OXYCOCCANA* (DIPTERA: CECIDOMYIIDAE), AND ITS HOST PLANT, CULTIVATED CRANBERRY (*VACCINIUM MACROCARPON*)

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SUNIL TEWARI, B.S., G. B. P. U. A. & T, PANTNAGAR, INDIA

M.S., LOUISIANA STATE UNIVERSITY, BATON ROUGE

Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Anne L. Averill

Cranberry tipworm, *Dasineura oxycoccana* Johnson (a gall-making fly), disrupts normal growth of cranberry (*Vaccinium macrocarpon* Aiton) by injuring the apical meristem of shoots or uprights. The impact of larval feeding injury on reproductive parameters of cranberry was determined, from one growing season to next, at upright (Maine and Massachusetts, 2008 - 2009) and plot levels (Massachusetts, 2009 – 2010 and 2010 - 2011). I also estimated the proportions of uprights injured because of tipworm feeding at several cranberry production sites (Massachusetts and Maine) and the proportions of uprights that produced flowers and fruits in the next growing season. Tipworm-injured uprights tagged at the end of the growing season did not produce floral-units (following year) across sites in both Massachusetts and Maine. There was significant variation among the sampled sites in the proportions of tipworm-injured uprights and also in the proportions of uprights with flowers in the next growing season (Massachusetts and Maine). A trend was apparent wherein sites with higher tipworm injury levels had relatively lower flowering proportions in the next growing season. However, sites in

Massachusetts did not differ in the proportions of uprights that set fruit and in a replicated study, significant reduction in tipworm injury at plot level (using insecticide) did not impact flower and fruit production in the next growing season. A two-year field study was carried out at three different locations to determine the impact of tipworm feeding injury on the reproductive and vegetative growth of two cranberry cultivars ('Howes' and 'Stevens') in Massachusetts. Individual uprights of cranberry exhibited tolerance to natural (tipworm) and simulated apical meristem injury in the current growing season (fruit production) and results were corroborated by a greenhouse study. In the field study, weight of fruit was higher in tipworm-injured uprights as compared with intact control uprights at the sites with Howes. However, majority of injured uprights (tipworm and simulated) did not produce new growth from lateral buds (side-shoots) before the onset of dormancy. In the next growing season, fewer injured uprights resumed growth and produced flowers as compared with intact uprights at two of the three sites.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INJURY TO APICAL MERISTEM OF CRANBERRY BY *DASINEURA OXYCOCCANA* (DIPTERA: CECIDOMYIIDAE) REDUCES PRODUCTION OF FLORAL-UNITS IN THE NEXT GROWING SEASON

Introduction

Apical meristems of plants play an important role in growth and are susceptible to damage by a number of abiotic (fire, frost, and wind) and biotic (vertebrate and invertebrate herbivores) factors, with even minor injury having a profound impact on fitness (Wise and Abrahamson 2008). Apical meristems are particularly vulnerable to boring/gall-inducing insects as a result of being tender and having higher concentrations of mineral nutrients than the other plant tissues (Wise and Abrahamson 2008). In perennial hosts, feeding by galling insects can impact reproduction in the subsequent growing seasons as a result of altered plant architecture and potential reduction in the number of flower bearing shoots (Martinez et al. 1992, DeClerck-Floate and Price 1994, Gonzales et al. 2005). However, many perennial plants are able to compensate for the injury to apical meristems (natural and artificial) by producing lateral shoots from axillary buds (Whitham and Mopper 1985, Marquis 1996, Wilson 2000, Nakamura and Ohgushi 2007).

The cultivated cranberry is a perennial, low-growing woody vine native to North America (Vander Kloet 1988). Horizontal stolons, also known as runners, colonize the soil surface and bear vertical shoots called uprights. Uprights are terminated by a mixed bud and in the next growing season these either produce

vegetative growth only (vegetative upright), or vegetative growth with blossoms/floral-units in the basipetal positions (flowering upright) (Eck 1990) (Fig. 1). The growth form of an upright, i.e. vegetative or flowering, depends upon the initiation of flowering meristem that begins in late June or early July of the previous growing season (Eaton 1978) (Fig. 2). Cranberry has been commercially cultivated in the United States for ≈200 years and most of the early cultivars were selections from wild native stands (Dana 1983, Roper 2001). Cultivar 'Howes' is an example of one such native selection that is common in MA. A breeding program for cranberry was initiated in 1929 by the United States Department of Agriculture (USDA) in collaboration with the University of Massachusetts Cranberry Experiment Station (East Wareham, MA), and Rutgers University (NJ). The primary focus of the program was to breed resistance against false blossom disease (Roper 2001), a condition caused by a phytoplasma and vectored by the blunt-nosed leafhopper (*Euscelis striatulus* Fallen) (Chen 1971). 'Stevens', a preferred cultivar in most of the cranberry growing regions, was an outcome of this breeding program (Roper 2001). In terms of phenology and relative fruit size, Stevens matures early and produces bigger fruits as compared with the late maturing and small-fruited Howes. 'Crimson Queen' is a high-yielding hybrid cultivar ('Ben Lear' × Stevens) released recently from Rutgers University and is being adopted by MA growers. Cranberry is commercially cultivated as a fruit crop in Massachusetts, New Jersey, Oregon, Washington, Wisconsin (Eck 1990) and more recently in Maine. In Canada, British Columbia and Quebec are the major production regions of cranberry in terms of acreage (Agriculture and Agri-Food Canada 2009).

Tipworm is a tiny gall midge ≈2.0 mm long (Mahr 2005) and injury caused by larval feeding results in death of apical meristem (uprights and runners) in cranberry. Injured uprights may resume vegetative growth by producing side-shoots from lateral axillary buds in the same or subsequent growing seasons (Voss 1996) (Fig. 1). Cranberry tipworm overwinters as late instar larvae (Gagne 1989) and flies emerge around mid-May in MA. Tipworm adults live for ≈4-6 d and spend majority of time protected among the cranberry vines (Smith 1890). Females deposit one or more eggs on the apical meristem tissue of an actively growing cranberry shoot. The larvae feed on plant juices by piercing through the meristematic tissue (Cook et al. 2011), and undergo three instars while developing in a cup shaped gall (Gagne 1989, Cook et al. 2011). The life cycle from eggs to adults takes10-15 d and there can be multiple overlapping generations in a single growing season (Gagne 1989). It has been suggested that the length of the growing season is one of the factors that determines the impact of tipworm injury on commercial cranberry production (Mahr and Perry 2006). Mahr and Perry (2006) hypothesized that cranberry growing regions in the northern latitudes with a shorter growing season will be more adversely impacted by tipworm injury than those in southern latitudes. Tipworm has been of special concern to the cranberry growers in Maine and is perceived as one of the most important insect pests (Charles Armstrong, personal communication). More recently, tipworm has reached pest status in the cranberry growing regions of British Columbia, Canada (Cook et al. 2011).

The main goal of this study was to determine the flowering response, in the next growing season, of individual cranberry uprights with tipworm feeding injury.

Since biennial bearing has been reported in cranberry (Roper et al. 1993), we studied both flowering and vegetative uprights in three cranberry cultivars and at two separate geographical regions (Massachusetts and Maine). We carried out two additional studies to interpret and discuss results of the main investigation in a wider context. First was a multi-site sampling study done in 2009-2010 at commercial cranberry plantings (Massachusetts and Maine) and the purpose was to estimate: 1) The proportions of uprights injured because of tipworm feeding and variation in injury levels among sites, 2) the proportion of uprights that produced flowers and set fruit at the same sites in the next growing season and variation in these variables among the sites. The sampled sites were representative of commercial production areas in the two regions (Massachusetts and Maine) and included the two common cultivars (Howes and Stevens). The overall goal of the sampling study was to quantify end of season tipworm injury and identify the potential factors that may explain variation in injury levels among sites and also between the two growing regions (Massachusetts and Maine). We were also interested in detecting patterns between variation in tipworm injury and flower or fruit production across the sampled sites. In the second study a systemic insecticide was used to determine the impact of tipworm injury suppression, at plot level, on flower, and fruit production in the next growing season (Massachusetts). The study was carried out on cultivar Howes in 2009 - 2010 and 2010 - 2011.

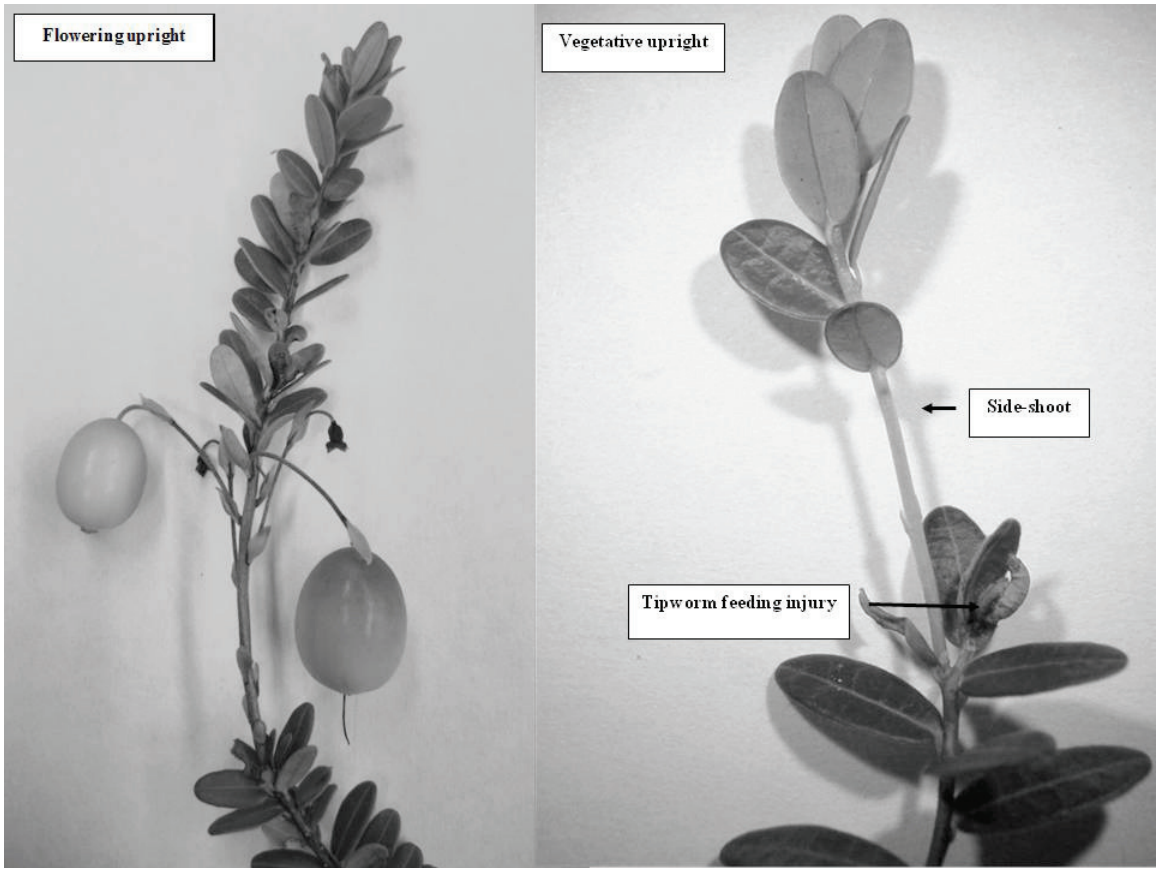

Figure 1. A flowering cranberry upright with mature fruits and a vegetative upright with tipworm feeding injury and a side-shoot, which was produced after death of apical meristem.

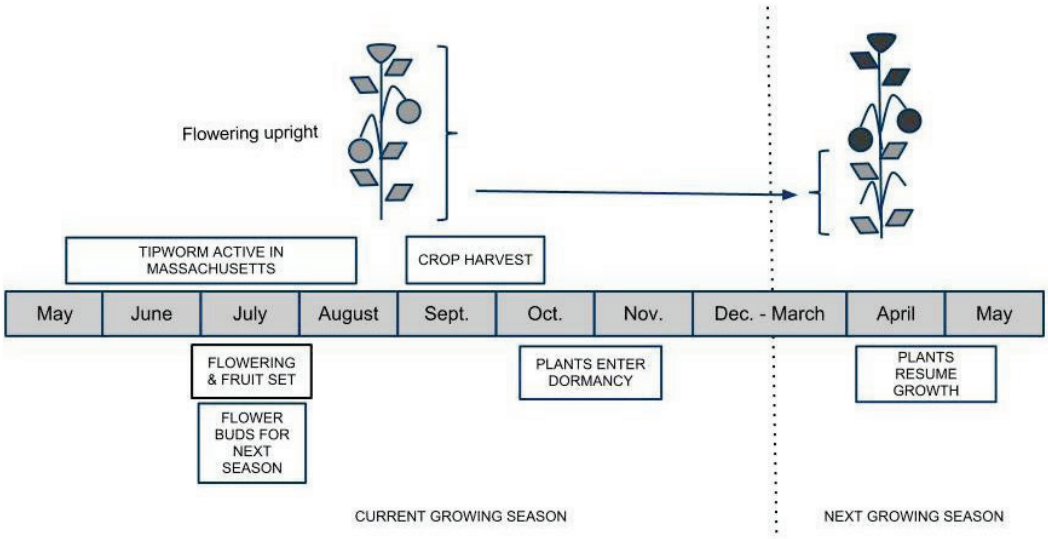

Figure 2. The 12-mo growth cycle of cranberry in Massachusetts.

Materials and Methods

Upright-level Effect: Tagging Study

We tagged individual cranberry uprights (flowering and vegetative) that exhibited tipworm feeding injury and intact control uprights at sites across the growing regions of Massachusetts and Maine. The goal of this study was to determine the impact of tipworm injury on production of floral-units in the next growing season. Unopened flowers, fully developed flowers, and developing fruits were all classified as floral-units because we did not distinguish between them at the time of data collection.

General methods. One research plot per site was established after harvest (October 2008) in both Massachusetts and Maine. The age of plantings at each site

were as follows: MA1 – 4 yr, MA2 – 7 yr, MA3 – 50 yr, MA4 – 2 yr, MA5 – 10 yr, MA6 – 9 yr, ME1 – 10 yr, ME2 – 10 yr, ME3 – 6 yr, and ME4 – 10 yr. All the sites were commercial cranberry beds and in active production, except MA4. At site MA4, the bed was flooded at full bloom to dislodge flowers, a cultural practice that encourages vegetative growth and is common in new plantings. We outlined an area of 0.4 ha at each site (plot) and five transects, each 5 m long, were set up equidistant from one another to cover the entire plot. The transects were laid down perpendicular to the direction in which the majority of runners were oriented to avoid tagging more than one upright on a single runner. Along each transect, 40 uprights belonging to four different types (10 each) were tagged for a total of 200 uprights per site (40/transect x 5 transects): a) flowering uprights showing tipworm injury (FI) b) flowering uprights free of tipworm injury (FNI) c) vegetative uprights showing tipworm injury (VI) d) vegetative uprights free of tipworm injury (VNI). Presence of persistent pedicles called hooks was used to identify flowering uprights (Strik et al. 1991, Roper et al. 1993). Plastic cable ties of four different colors were used to tag the four classes of uprights. In addition, a small metal disc with a unique number to identify individual uprights was also attached to the cable tie. Tagging of uprights type began at one end of the transect and terminated at the other end with all four types covering approximately the same length of the transect. The length of transects varied across sites, depending upon upright injury levels, but was consistent within a site. The tagged uprights were checked for the presence or absence of floral-units in June 2009.

*Massachusetts***.** Plots were established at six sites (MA1: 41°45'27.43" N 70°45'25.14" W, MA2: 41°50'25.63" N 70°44'06.26" W , MA3: 41°47'31.38" N 70°46'59.71" W, MA4: 41°45'58.29" N 70°40'10.01" W, MA5: 41°53'53.39" N 70°43'43.55" W, and MA6: 41°53'07.96" N 70°41'52.53" W) representing three cultivars (Crimson Queen, Howes, and Stevens), with each cultivar tagged at two sites (Crimson Queen – MA1 and MA2, Howes – MA3 and MA4, Stevens – MA5 and MA6).

*Maine***.** Plots were established at four sites (ME1: 44°17'49.01" N 69°07'13.31" W, ME2: 45°07'02.71" N 67°09'03.48" W, ME 3: 44°13'17.22" N 69°43'59.45" W, and ME4: 44°04'23.10" N 69°45'43.00" W) representing two cultivars (Howes and Stevens). Cultivar Howes was tagged at one site (ME1) and Stevens at three sites (ME2, ME3, and ME4).

Site-level Effect: Sampling Study

We collected samples of uprights from multiple commercial cranberry plantings in Massachusetts and Maine to estimate the proportions of uprights exhibiting tipworm feeding injury at the end of the growing season. Samples were also collected from the same sites in the next growing season to estimate proportions of uprights with flowers and fruits. The goal of this study was to quantify end of season tipworm injury across the sites in the two regions (Massachusetts and Maine) and to detect trends linking flower or fruit production at the sampled sites to previous season variation in the injury levels.

*Massachusetts***.** In November 2009, we collected cranberry uprights from commercial cranberry plantings at five different sites (MA3, MA5, MA6, MA7: 41°56'54.04" N 70°47'28.79" W, and MA8: 41°56'28.21" N 70°40'34.84" W), including three sites used in the tagging study (MA3, MA5, and MA6). Sites MA7 (Howes – 50 yr) and MA8 (Stevens – 5 yr) were both commercial plantings and in active production. At each site, a metal ring (132 cm^2) was placed on the cranberry bed and all the enclosed uprights were collected. A total of five samples were collected from 0.2 ha plot at each site by walking haphazardly to a section of the sampling area, dropping the metal ring some distance in front, and collecting all the enclosed uprights. The samples were brought back to the laboratory and examined to estimate the proportions of uprights with apical meristem injured due to tipworm feeding. In September 2010, we collected samples from the same sites using identical protocol and examined the uprights to estimate the proportions with flowers and those with one or more fruits. The uprights that aborted all the flowers or only produced under-developed fruits (pin-heads) were not counted in the estimation of the fruiting proportions.

*Maine***.** In November 2009, we collected uprights from commercial cranberry plantings at four different sites (ME1, ME2, ME5: 44°16'15.56" N 70°16'59.82" W, and ME6: 44°42'06.85" N 67°58'21.69" W), including two sites used in the tagging study (ME1 and ME2) to estimate proportions of uprights with apical meristem injured due to tipworm feeding. Sites ME5 (11 yr) and ME6 (9 yr) were commercial plantings (both cultivar Stevens) and in active production. In August 2010, we collected samples from the same sites to estimate proportions of uprights with

flowers and those with one or more fruits. The size of the plots and the sampling protocol were identical to those used in Massachusetts.

Plot-level Effect: Suppression Study

We carried out a plot-level study in Massachusetts to determine the impact of tipworm injury suppression, for one growing season, on flower and fruit production in the following year. The goal of this study was to make a direct comparison between the plots in which tipworm feeding injury was suppressed using an insecticide, and the untreated control plots (as opposed to the site-level study in which the goal was to detect trends linking flower or fruit production at the sampled sites to previous season variation in tipworm injury levels).

The study was done on two different sections of a 50 yr old commercial Howes planting (41°56'54.51" N 70°47'27.99" W) in 2009 and 2010. Twelve plots, each measuring 3.81 m^2, were established (May 2009 and 2010) at the study site in a grid like pattern and a space of 5 m was maintained between them. We used technical grade systemic compound Movento (Bayer CropScience, Research Triangle Park, NC), which causes tipworm larval mortality upon ingestion, to suppress feeding injury inside the plots. For the study we used two manufacturer recommended application rates (350 and 700 ml/ha), with a spray volume of 448 liters/ha. We are not aware of any published reports that document the direct impact of Movento on growth and reproduction in cranberry plants. The study was a completely randomized design with four replicates (plots) per treatment (two insecticide application rates and a control). The study began on first week of June in

2009 and third week of May in 2010 and application of insecticide was repeated approximately every 12 days (depending upon local weather conditions) using a CO_2 powered backpack sprayer. Application was terminated once tipworm life-stages (eggs, larvae, and pupae) could not be detected in samples of uprights collected from the plots each month. We estimated the end of season tipworm injury levels by collecting upright samples from the plots in October 2009 and 2010. One sample per plot was collected from each of the 12 plots by walking haphazardly to a section of the plot, dropping a metal ring (132 cm^2) some distance in front and collecting all the enclosed uprights. Individual uprights from each sample were checked in the laboratory for signs of tipworm feeding injury. The plots were left untreated in the next growing season and flower/fruit production was estimated by collecting upright samples in August 2010 and 2011. The variables measured were, 1) number of uprights per sample with flowers, 2) number of uprights per sample with fruit, and 3) total number of fruit per sample. All berries having a diameter of 0.5 cm or more were classified as fruit. Sample collection protocol was identical to the one used in the previous growing season to estimate the injury levels. In October 2010 and 2011, we collected three foot-square samples of fruit per plot using a metal frame to estimate yield. Harvested berries were brought back to the laboratory in coolers and fresh weight of each sample was determined.

Data analysis

Upright-level Effect: Tagging Study. We used a χ^2 based categorical data analysis model (PROC FREQ; SAS Institute 2009) with type of upright (FI, FNI, VI,

and VNI) as the explanatory variable to determine the overall impact of tipworm feeding injury on production of floral-units (presence or absence) at each site in the two growing regions (Massachusetts and Maine). Additionally, we also carried out separate analysis comparing injured and non-injured uprights by their growth form in the previous season (flowering and vegetative). We compared the two types of injured uprights (FI and VI) at each site to determine whether or not the effect of injury on floral-unit production varied based on growth form (flowering or vegetative) in the previous season. A similar analysis was done to compare the response of non-injured uprights (FNI and VNI) at each site. For each of the four types of uprights, χ^2 tests were used to compare all the sites within each growing region (Massachusetts and Maine) and the two sites for each of the three cultivars in Massachusetts and the two sites for cultivar Stevens in Maine. We also determined the impact of transect on production of floral-units by comparing the four types of tagged uprights across the five transects at each of the site-cultivar combinations in both Massachusetts and Maine.

One of the transects at sites MA4 and ME1 were found in a waterlogged section of the cranberry planting at the time of data collection (June 2009) and their results were not included in the analysis. Early season frost injury to the growing uprights disrupted production of floral-units at one of the sites (ME4) and it was excluded from data analysis.

Site-level Effect: Sampling Study. We used Proc Surveymeans (SAS Institute 2009) to estimate the proportions of tipworm-injured uprights, flowering uprights, and fruiting uprights with their associated standard errors at each site-state in the

sampling study. This test views the pair (e.g. number of uprights in a sample, number of uprights injured due to tipworm feeding) as random and accounts for the difference in the number of uprights collected in the five samples per site. Proc Iml (SAS Institute 2009) was used to construct a test of equal proportions for comparing sites within each growing region (Massachusetts and Maine). This test utilized the previously estimated proportions and their associated standard errors in a general χ^2 test. Correlation analysis (SAS Institute 2009) was used to test for the type and strength of relationship between the proportions of uprights with tipworm injury (2009) and the proportions of uprights with flowers at the same sites in the following year (2010) (Massachusetts and Maine). Similar analyses were carried out to determine the type and strength of relationship between the proportions of uprights with flowers and those with one or more fruits (2010) at each site (Massachusetts and Maine). For all correlation analyses, Spearman coefficients are reported in the results section.

Plot-level Effect: Suppression Study. The two insecticide application rates were equally effective in suppressing tipworm feeding injury (data not presented) and we only compared plots that received Movento at 700 ml/ha to the control plots for the different variables of interest (see Materials and Methods). We used Proc Surveymeans and Proc Iml (SAS Institute 2009) to estimate and compare proportions between the treated and control plots for tipworm injury, flowering, and fruit production. Proc Npar1way (SAS Institute 2009) was used to compare the number of fruits per sampling unit between the treated and control plots in a nonparametric test. Analysis of variance (ANOVA) (PROC GLM; SAS Institute 2009)

with plot as a random factor and nested within treatment was used to compare the fresh weight of fruits harvested from the treated and control plots.

Results

Transect did not have a significant impact on the production of floral-units for the four types of tagged uprights at all the sites in both Massachusetts and Maine (upright-level tagging study) and subsequent analyses do not assume a transect structure.

Upright-level Effect: Tagging study (Massachusetts)

Compared with intact cranberry uprights, fewer uprights with apical meristem injured due to tipworm feeding (2008) produced floral-units in 2009. This result was significant at all the sites regardless of the cultivar and also for the two growth forms (flowering and vegetative) (Tables 1 and 2). There was no difference in the production of floral-units between injured flowering and injured vegetative uprights at all the six sites (Table 2). Similarly, there was no difference in production of floral-units between non-injured flowering and non-injured vegetative uprights at the six sites (Table 2). Uprights that were injured (both flowering and vegetative) at the end of the 2008 growing season and also did not produce floral-units in 2009 ranged between 94 and 100% at the six sites (Table 1). Similarly, for the uprights that were not injured, those without floral-units in 2009 ranged between 4 and 21% at the same six sites (Table 1). For each of the four types of uprights, production of floral-units did not vary across the six sites except for VNI (Table 3). However, on

comparing the two sites with the same cultivar, only Stevens sites showed difference in the flowering response of the type VNI (Table 3) and not the sites with Crimson Queen and Howes (Table 3).

Table 1. Proportion of flowering (F) and vegetative (V) tagged uprights (mean ± SE), injured (I) and not injured (NI) by tipworm feeding in 2008, that had no floral-units in June 2009 (Massachusetts and Maine).

	Injured in 2008		Not injured in 2008	
	FI	VI	FNI	VNI
Site (Cultivar)				
MA1 (C. Queen)	1 (0)	0.98 (0.02)	0.09 (0.04)	0.06 (0.03)
MA2 (C. Queen)	0.98 (0.02)	0.98 (0.02)	0.06 (0.03)	0.04 (0.03)
MA3 (Howes)	1 (0)	1 (0)	0.06 (0.03)	0.14 (0.05)
MA4 (Howes)	1 (0)	0.97 (0.02)	0.15 (0.05)	0.10 (0.05)
MA5 (Stevens)	1 (0)	0.94 (0.03)	0.10 (0.04)	0.21 (0.06)
MA6 (Stevens)	1 (0)	0.98 (0.02)	0.08 (0.03)	0.04 (0.03)
ME1 (Howes)	1 (0)	0.97 (0.02)	0.57 (0.08)	0.28 (0.07)
ME2 (Stevens)	1 (0)	1 (0)	0.44 (0.07)	0.21 (0.06)
ME3 (Stevens)	1 (0)	0.97 (0.02)	0.12 (0.04)	0.29 (0.07)

Table 2. Comparisons of the flowering response (2009) for the four different types of tagged uprights (2008) at each of the six sites in Massachusetts, and the three sites in Maine.

Site (Cultivar)	All four types	FI vs. FNI[1]	VI vs. VNI	FI vs. VI	FNI vs. VNI
MA1 (C. Queen)	146.15 (3), $p < 0.0001$[2]	71.27 (1), $p < 0.0001$	74.83 (1), $p < 0.0001$	0.98 (1), $p = 0.32$	0.24 (1), $p = 0.62$
MA2 (C. Queen)	157.25 (3), $p < 0.0001$	77.75 (1), $p < 0.0001$	79.43 (1), $p < 0.0001$	0.003 (1), $p = 0.95$	0.26 (1), $p = 0.60$
MA3 (Howes)	151.67 (3), $p < 0.0001$	85.70 (1), $p < 0.0001$	65.87 (1), $p < 0.0001$	-[3]	1.97 (1), $p = 0.15$
MA4 (Howes)	146.15 (3), $p < 0.0001$	55.33 (1), $p < 0.0001$	56.67 (1), $p < 0.0001$	0.98 (1), $p = 0.32$	0.34 (1), $p = 0.55$
MA5 (Stevens)	132.19 (3), $p < 0.0001$	70.81 (1), $p < 0.0001$	51.16 (1), $p < 0.0001$	3.22 (1), $p = 0.07$	2.23 (1), $p = 0.13$
MA6 (Stevens)	158.71 (3), $p < 0.0001$	80.26 (1), $p < 0.0001$	78.43 (1), $p < 0.0001$	1.03 (1), $p = 0.30$	0.54 (1), $p = 0.46$
ME1 (Howes)	63.14 (3), $p < 0.0001$	20.26 (1), $p < 0.0001$	37.60 (1), $p < 0.0001$	1.01 (1), $p = 0.31$	6.36 (1), $p = 0.01$
ME2 (Stevens)	96.31 (3), $p < 0.0001$	35.23 (1), $p < 0.0001$	57.60 (1), $p < 0.0001$	-[3]	5.61 (1), $p = 0.02$
ME3 (Stevens)	124.21 (3), $p < 0.0001$	73.66 (1), $p < 0.0001$	44.32 (1), $p < 0.0001$	1.07 (1), $p = 0.29$	3.66 (1), $p = 0.06$

[1] FI = Flowering and injured, FNI = Flowering and not injured, VI = Vegetative and injured, and VNI = Vegetative and not injured.

[2] Chi-square test values, degrees of freedom (in parentheses), and p values are reported for each test.

[3] None of the uprights produced floral-units.

Table 3. Comparisons among the sites in Massachusetts (2009) for the flowering response of the four types of tagged uprights (2008).

Type of Upright	All Six Sites	C. Queen Sites	Howes Sites	Stevens Sites
FI[1]	4.60 (5), p = 0.465[2]	0.92 (1), p = 0.335	-[3]	-[3]
FNI	2.80 (5), p = 0.730	0.24 (1), p = 0.622	2.02 (1), p = 0.154	0.14 (1), p = 0.702
VI	3.67 (5), p = 0.596	0.0002 (1), p = 0.987	1.14 (1), p = 0.284	0.87 (1), p = 0.350
VNI	11.12 (5), p = 0.048	0.26 (1), p = 0.609	0.30 (1), p = 0.581	5.92 (1), p = 0.014

[1] FI = Flowering and injured, FNI = Flowering and not injured, VI = Vegetative and injured, and VNI = Vegetative and not injured.

[2] Chi-square test values, degrees of freedom (in parentheses), and p values are reported for each test.

[3] None of the uprights produced floral-units.

Upright-level Effect: Tagging Study (Maine)

Like Massachusetts, significantly fewer uprights with tipworm feeding injury (2008) produced floral-units in the next growing season (2009), as compared with the intact uprights. This result was significant at all three sites regardless of the cultivar and also for the two growth forms (flowering and vegetative) (Tables 1 and 2). Also, like MA, there was no difference in the production of floral-units between injured flowering and injured vegetative uprights at the three sites (Table 2). However, unlike MA, a greater number of non-injured vegetative uprights produced floral-units as compared with the non-injured flowering uprights at sites ME1 and ME2 (Table 2). Uprights that were injured (both flowering and vegetative) at the end of the 2008 growing season and also did not produce floral-units in 2009 ranged between 97 and 100% at the three sites (Table 1). Similarly, for the uprights that were not injured, those without floral-units in 2009 ranged between 12 and 57% at the same three sites (Table 1). For each of the four types of uprights, production of floral-units did not vary across the three sites except for FNI (Table 4). Also, the two Stevens sites were different in production of floral-units for the upright type FNI (Table 4).

Table 4. Comparisons among the sites in Maine (2009) for the flowering response of the four types of tagged uprights (2008).

Type of Upright	All Three Sites	Stevens Sites
FI[1]	-[2]	-[2]
FNI	20.04 (2),	11.37 (1),
	p < 0.0001[3]	p = 0.0007
VI	1.12 (2)	1.01 (1)
	p = 0.570	p = 0.314
VNI	0.55 (2)	0.46 (1)
	p = 0.758	p = 0.495

[1] FI = Flowering and injured, FNI = Flowering and not injured, VI = Vegetative and injured, and VNI = Vegetative and not injured.

[2] None of the uprights produced floral-units.

[3] Chi-square test values, degrees of freedom (in parentheses), and p values are reported for each test.

Site-level Effect: Sampling Study (Massachusetts)

The end of season tipworm injury levels varied significantly across the sites (χ^2 = 108.97; df = 4; p < 0.0001) in 2009. The two sites with cultivar Howes had 36 and 40% of uprights injured as compared with 3, 4, and 13% of uprights injured at the sites with cultivar Stevens (Fig. 3). In the next growing season, we documented variation among the same sites in the proportions of uprights with flowers (χ^2 =

30.85; df = 4; $p < 0.0001$). However, there was no difference among the sampled sites in the proportions of uprights that set one or more fruits ($p > 0.05$). Site MA5, with numerically lowest tipworm-feeding injury recorded in 2009 (3%), had the highest proportion of uprights with flowers (53%) in 2010 (Fig. 3). Also, MA3 with the highest feeding injury recorded in 2009 (40%) had only 28% of the uprights produce flowers in 2010, the lowest of all the sampled sites. The correlation between the proportions of uprights with tipworm injury (2009) and proportions of uprights at the same sites with flowers in the next growing season (2010) was negative, but not significant ($p > 0.05$). We recorded a positive and significant correlation between the proportions of uprights with flowers and the proportions of uprights in the same sample that set one or more fruits at sites MA3 ($r(3) = 0.97$, $p = 0.0048$), MA6 ($r(3) = 0.97$, $p = 0.0048$), and MA7 ($r(3) = 0.94$, $p = 0.0138$) in 2010 (Fig. 4). However, although positive, the correlation between flowering and fruiting was not significant ($p > 0.05$) at sites MA5 and MA8 (both cultivar Stevens). Also, the flowering proportions recorded at sites MA5 and MA8 were numerically higher than the three other sites sampled in MA (Fig. 4).

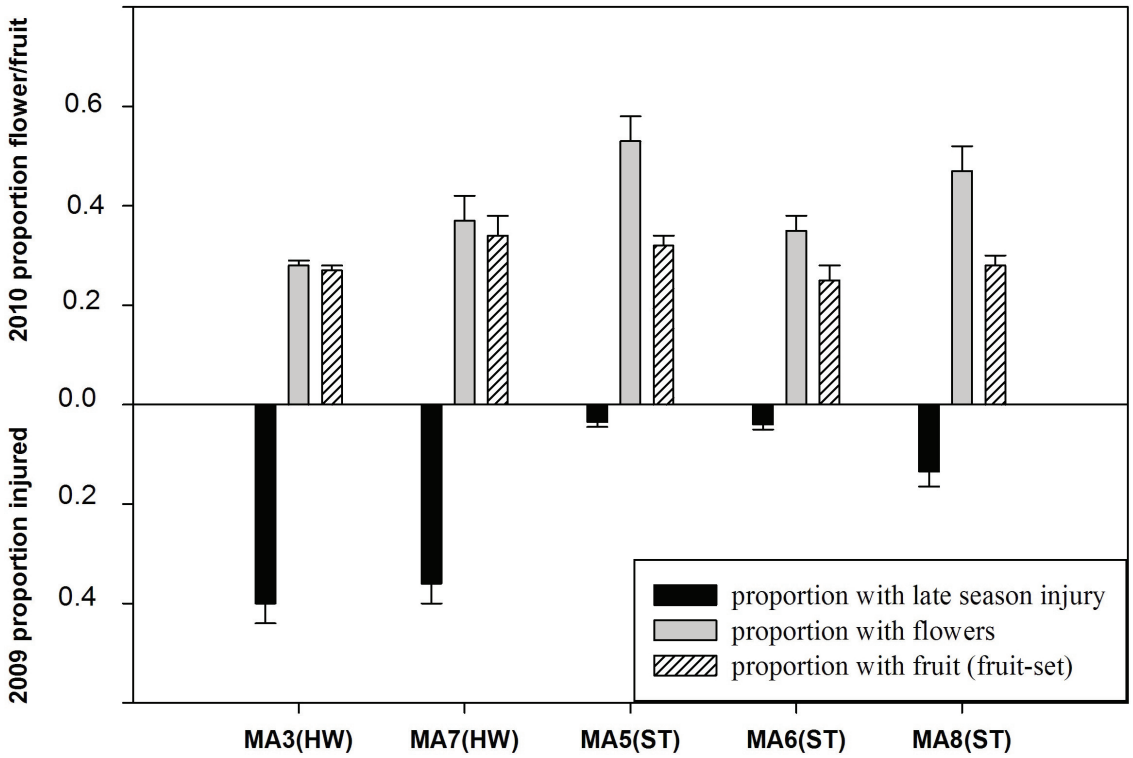

Figure 3. Estimated proportions (mean, SE) of uprights with injury (2009) (lower figure section); proportion uprights flowering and uprights with one or more mature fruits (both 2010) (upper figure section) at different sites in Massachusetts. Two cultivars were evaluated, Howes (HW) and Stevens (ST).

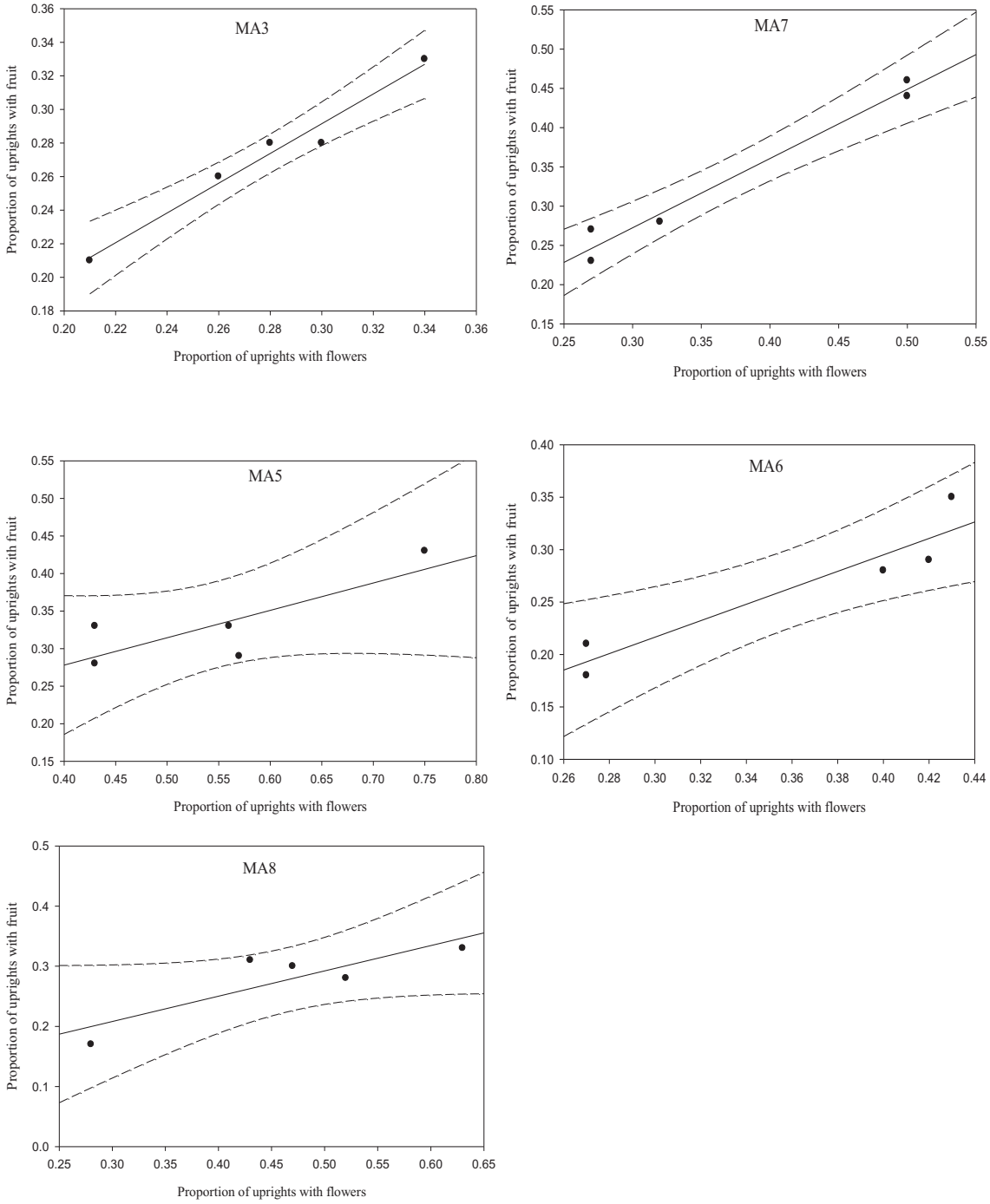

Figure 4. First order regression lines (with 95% CI) showing positive relationship between proportions of uprights with flowers and proportions of uprights with one or more mature fruits at different sites in Massachusetts (Howes: MA3 and MA7; Stevens: MA5, MA6, and MA8). Relationship significant at sites MA3, MA6, and MA7.

Site-level Effect: Sampling study (Maine)

Like Massachusetts, tipworm injury levels at the end of the 2009 growing season varied across the sites ($\chi^2 = 98.04$; df = 3; $p < 0.0001$). The site with cultivar Howes had 53% of the uprights injured as compared to 13, 30, and 48% of uprights injured at the sites with cultivar Stevens (Fig 5). However, unlike Massachusetts, the sites varied in the proportions of uprights with flowers as well as fruits in the next growing season ($\chi^2 = 17.37$; df = 3; $p = 0.0005$, $\chi^2 = 20.16$; df = 3; $p = 0.0001$). Of all the sites sampled in 2009, the numerically lowest tipworm injury was recorded at ME6 (12%) (Fig. 5). At site ME6, we also recorded numerically highest proportion of uprights with flowers and fruits (both 25%) in the next growing season (Fig. 5). Like Massachusetts, the negative correlation between the proportions of uprights with tipworm injury (2009) and the proportions of uprights at the same sites with flowers in the next growing season (2010) was not significant ($p > 0.05$). We recorded a positive and significant correlation between the proportions of uprights with flowers and the proportion of uprights in the same sample that set one or more fruits at sites ME1 ($r(3) = 0.97$, $p = 0.005$) and ME6 ($r(3) = 0.94$, $p = 0.014$) in 2010 (Fig. 6). However, although positive, the correlation between flowering and fruiting was not significant at sites ME2 and ME5 (both cultivar Stevens) ($p > 0.05$) (Fig. 6).

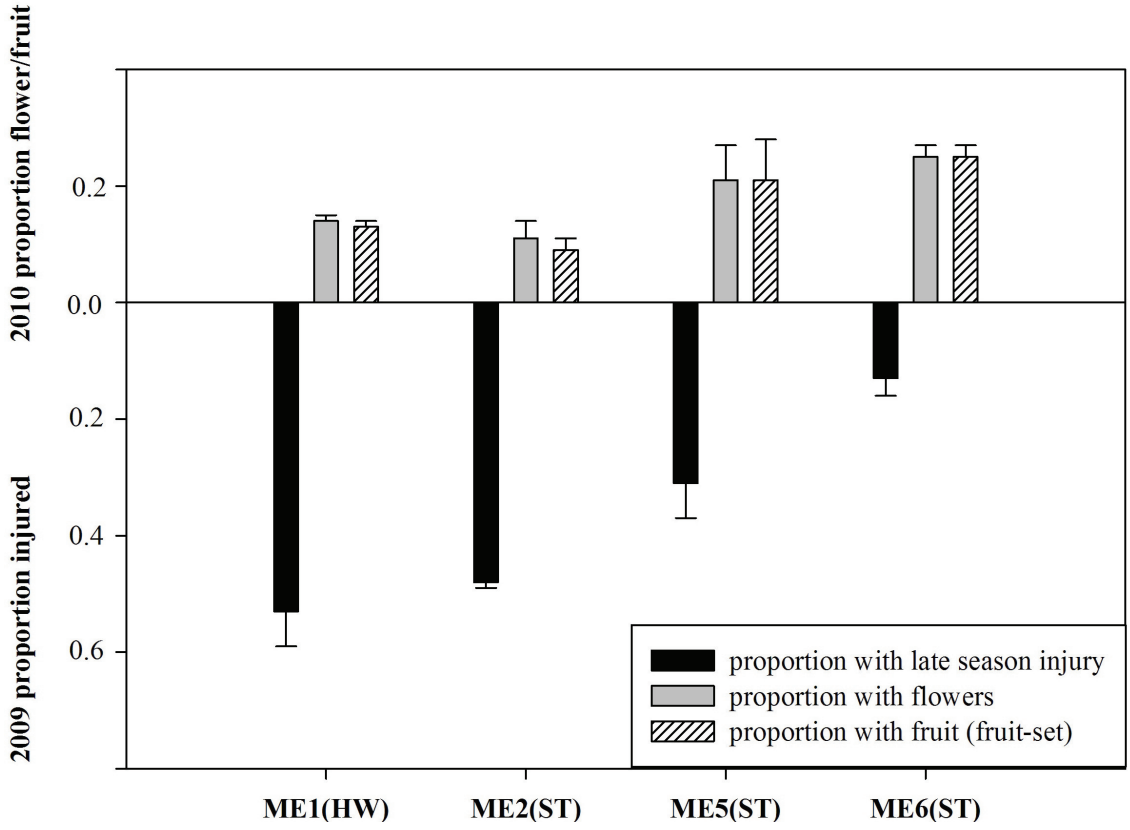

Figure 5. Estimated proportions (mean, SE) of uprights with injury (2009) (lower section figure); proportion uprights flowering and uprights with one or more mature fruits (both 2010) (upper figure section) at different sites in Maine. Two cultivars were evaluated, Howes (HW) and Stevens (ST).

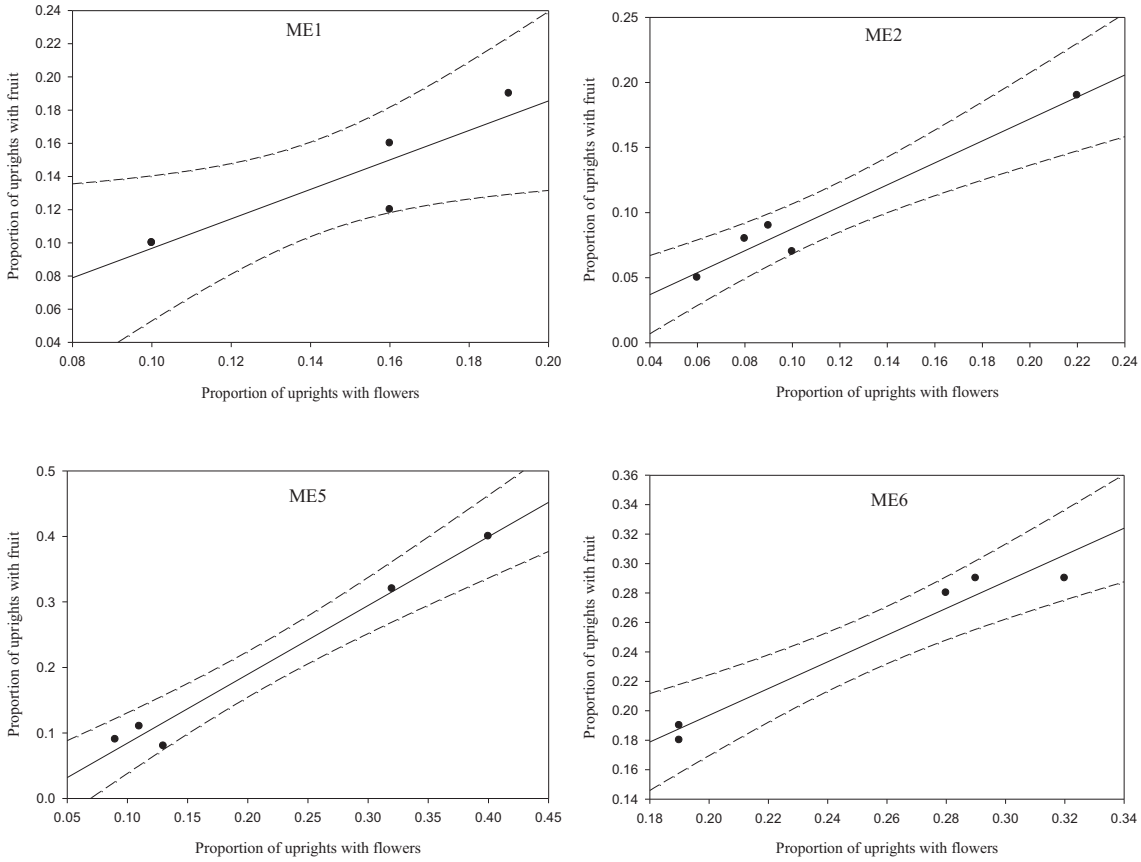

Figure 6. First order regression lines (with 95% CI) showing positive relationship between proportions of uprights with flowers and proportions of upright with one or more mature fruits at different sites in Maine (Howes: ME1; Stevens: ME2, ME5, and ME6). Relationship significant at sites ME1 and ME6.

Plot-level Effect: Suppression Study

The insecticide-treated plots had fewer uprights with tipworm feeding injury as compared with the control plots in both 2009 and 2010 ($\chi^2 = 19.6$; df = 1; $p < 0.0001$, $\chi^2 = 20.39$; df = 1; $p < 0.0001$) (Table 5). The tipworm injury levels inside the control plots (36% in 2009 and 32% in 2010) were consistent with those recorded at the other Howes plantings in Massachusetts (S.T., unpublished data) and fewer

than 5% of the uprights were injured in the insecticide treated plots (Table 5). There was no difference between the treated and the control plots (in the next growing season) for the different variables measured (proportion of upright with flowers and fruits, number of fruits, and biomass of fruits) in both 2010 and 2011 ($p > 0.05$) (Table 5).

Table 5. Estimated proportions of tipworm-injured uprights and reproductive variables measured on the same plots in the next growing season between tipworm excluded (insecticide) and control treatments (mean ± SE).

	2009 Trial		2010 Trial	
Treatment/ Variable	Insecticide	Control	Insecticide	Control
Proportion injured[1§]	0.01 (0.01)	0.36 (0.07)	0.03 (0.02)	0.32 (0.05)
Proportion flowering	0.45 (0.06)	0.38 (0.01)	0.45 (0.05)	0.51 (0.05)
Proportion fruiting	0.4 (0.05)	0.35 (0.01)	0.39 (0.04)	0.43 (0.04)
Number of fruit[2]	53.75 (5.58)	47.0 (6.01)	61.25(8.87)	69.25 (6.92)
Fruit biomass[3]	250.12 (17.91)	224.39 (10.78)	271.91(22.58)	287.03 (22.11)

Discussion

The key finding of our study was that majority of cranberry uprights with tipworm feeding injury (95%) did not produce floral-units in the next growing

season. This result was consistent between the growth forms (flowering and vegetative) and the two regions (Massachusetts and Maine). In Massachusetts, tipworm are active from May to August and there can be multiple overlapping generations in a single growing season. In the tagging study, we did not differentiate the uprights based on when injury had occurred in the growing season. However, lack of variability in the flowering response of injured uprights (almost none produced floral-units) suggests that time of injury does not play a role in determining the growth form (flowering or vegetative) in the next growing season.

Although biennial bearing of flowers has been reported from the cranberry growing regions (Roper et al. 1993), we did not find evidence of a difference in the production of floral-units between non-injured vegetative and non-injured flowering uprights in Massachusetts. However, there was a difference in the production of floral-units between the non-injured flowering and non-injured vegetative uprights in Maine. Compared with non-injured flowering uprights, significantly more non-injured vegetative uprights produced floral-units at two of the three sites (ME1 and ME2) in the next growing season (2009). The tagging study also documented variation among sites (cultivar Stevens) in the production of floral-units for non-injured uprights in both Massachusetts (type VNI) and Maine (type FNI). Floral-unit production for the type VNI was numerically lowest at MA5 (MA) while it was highest for the type FNI at ME3 (ME). Roper et al. (1993) reported regional differences in flowering and fruiting response of cranberry uprights and concluded that climate and management practices may have played a role. However, site-specific factors such as physiological stress related to water and nutrient

availability at the time of floral meristem initiation, and frost injury to uprights early in the growing season may have also contributed to the variation in the production of floral-units among our study sites. We recorded a relatively wider range in the proportion of non-injured uprights that produced floral-units at the Maine sites (43 - 88%), as compared with the sites in Massachusetts (79 - 96%). Environmental conditions play a role in the balance between vegetative and reproductive growth in perennial plants (Brown and McNeil 2006). The relatively shorter growing season in ME (discussed in greater depth below) may be one of the environmental factors responsible for the lower rate of floral-unit production at the Maine sites, as compared with the sites in Massachusetts.

Uprights with tipworm feeding injury were collected from all the commercial cranberry production sites and we also documented variation among the sampled sites in the injury levels (Massachusetts and Maine) (site-level sampling study). Thus, we now have a better understanding of not only the occurrence (tipworm), but also the extent of its feeding injury and can identify some of the factors that may explain the variation among sites and also between the two growing regions. Marucci (1954) reported Howes to be the most susceptible cultivar in New Jersey and with an average tipworm infestation rate of 40%, a number consistent with our observations in Massachusetts (site-level sampling study). Compared with Howes, Stevens is an early season cultivar in terms of phenology and may explain results of the Massachusetts sampling study in which sites with Howes experienced more tipworm injury than Stevens. Uprights of phenologically advanced cranberry cultivars (e.g. Stevens) terminate growth relatively early in the season, which results

in the hardening of apical leaves and widening of the space between them. Tipworm females lay their eggs on actively growing shoots (Cockfield and Mahr 1994, Voss 1996) and in a late season cultivar like Howes, the uprights remain suitable for feeding by tipworm larvae comparatively later into the season. As a result, tipworm may be able to reach higher densities and complete more generations per season in cranberry plantings with cultivar Howes as compared with Stevens. The phenological window, a physiologically active period in the life cycle of plants most suitable for herbivores, is also critical for galling insects (Imai and Ohsaki 2006, Whipple et al. 2009). Boukili et al. (2007) reported that the establishment of a gall-midge on its host plant was positively related to stem growth. Vitou et al. (2008) reported that *Gephyraulus raphanistri* (Kieffer), a flower-bud galling insect, did not utilize *Brassica napus* L. in the field as a result of phenological asynchrony and not due to physiological or behavioral causes. Early emergence of flowers combined with the brief flowering period in *B. napus* excluded *G. raphanistri* from completing multiple generations, a result authors cited as evidence that host plant phenology can influence susceptibility to galling insects.

Compared with the results from the Stevens sites in Massachusetts, trend in data indicates higher proportions of tipworm-injured uprights at the two Stevens sites in Maine. This observation suggests that an environmental factor such as length of the growing season may impact the life history of both cranberry and tipworm and play a role in determining the injury levels. Climatic conditions exhibit a gradual change along the latitudinal gradient and the annual mean values of the cumulative daily radiation as well as the air temperature decline approximately

linearly with increasing latitude (Clevering et al. 2001). Maine has a shorter cranberry-growing season than Massachusetts and our sites in the two states were separated by about 4 degrees of latitude. In terms of cranberry growth phenology, the Maine sites included in this study are usually two weeks behind the cranberry growing region of MA, although the range can be from about a week to almost a month (Charles Armstrong, personal communication). Cranberry uprights can resume growth after tipworm feeding injury through the activation of lateral axillary buds (Fig. 1) and a shorter growing season could potentially impact the ability of the plant to produce side-shoots before the onset of dormancy. Huhta et al. (2000) reported that the southern population of the monocarpic herb *Gentianella campestris* (L.) was more tolerant of artificial shoot clipping than the northern one. The two populations were separated by about 5 degrees of latitude and the authors suggested that difference in the length of growing season might have impacted the ability of plants to compensate after injury. Whether the trend of high tipworm injury recorded at the Maine Stevens sites resulted from increased susceptibility due to altered life history/phenological window, differences in the production of side-shoots between the two growing regions (Massachusetts and Maine), or a combination of both is yet to be determined.

In the site-level sampling study (Massachusetts and Maine), trend suggests that sites with fewer tipworm-injured upright at the end of 2009 growing season had higher proportion of uprights with flowers in 2010. We were able to detect the negative effect of injury on floral-unit production (upright-level tagging study) as inter-site variation in flowering based on the previous season tipworm injury levels.

The one exception was site MA6 (Stevens) where we recorded relatively low proportion of uprights with flowers as compared with the two other Stevens sites (MA5 and MA8) with similar injury levels. However, although we documented variation among the Massachusetts sites in the proportions of uprights with flowers, the proportions of uprights that set one or more fruits were not different. Fruit set in cranberry is variable and approximately only half of floral-units in an upright produce mature fruit. Brown and McNeil (2006**)** suggested that the low fruit set in cranberry uprights due to abortion of distal flowers was most likely an ancestral trait and similar constraints may be associated with fruit set at a landscape level, especially since cranberry plants have evolved on acidic, sandy soils with little nutrient content (DeMoranville 2008). A two year study of flowering uprights reported similar trend wherein many flowers did not set viable fruit, especially in cultivars with high rate of return flowering (Strik et al. 1991). Total nonstructural carbohydrates (TNSC) in cranberry are at their lowest concentrations during fruit development and are also hypothesized to be responsible for low fruit set (Birrenkott et al. 1991, Hagidimitriou and Roper 1994). Our study documented a non-significant positive relationship between the uprights with flowers and those with one or more fruits at the Stevens sites in both Massachusetts and Maine (MA5, MA8, ME2, and ME5). This finding suggests that in a large fruited cultivar like Stevens, resource limitation may impose an upper bound on the total number of uprights that can successfully produce mature fruits. As a result the limited supply of resources may cause not just some, but all the flowers in an upright to abort fruit production. However, site-specific factors such as physiological stress related to

water and nutrient availability at the time of fruit set and injury from other herbivores and pathogens may have also played a role in the observed results for fruit set at the Massachusetts sites. The cranberry growing regions of Massachusetts experienced hot and dry weather conditions during the period coinciding with flowering and fruit set in 2010, a factor that may have negatively impacted the phenologically early cultivar Stevens at sites MA5 and MA8 (Fig. 3). In 2010, the air temperatures exceeded 32.2 C on eight different days during the months of July and August in East Wareham, MA (NOAA 2012). In comparison, the previous three years recorded either none or only two days of 32.2 C or higher temperatures for the same months (2007 – 0 d, 2008 – 2 d, and 2009 – 2 d) (NOAA 2012). Degaetano and Shulman (1987) reported that excessive heat from mid-June to early August was related to below normal cranberry production in New Jersey.

In the upright and site-level (tagging and sampling) studies, we did not directly compare the two states (Massachusetts and Maine) to determine whether or not the impact and extent of tipworm injury varied between the geographical regions because of qualitative differences between the sites in Massachusetts and Maine: 1) shorter growing season in Maine as compared with Massachusetts, 2) sites in Maine were geographically more spread out in contrast to the relatively clustered sites in Massachusetts, 3) plots were set up in relatively smaller commercial plantings in Maine as compared with Massachusetts. However, results from the upright-level tagging study indicate that tipworm injury inhibited the production of floral-units (next growing season) in both the states. Also, although our site-level sampling study demonstrates an overall trend of higher tipworm

injury levels and lower flower or fruit production rates at the sites in Maine as compared with Massachusetts; it does not provide direct evidence that the former is responsible for the latter. We were able to quantify the impact of tipworm injury suppression on the different reproductive parameters of cranberry (plot-level suppression study) and results indicate that flower and fruit production were not significantly affected by short-term control (discussed in greater depth below).

In the plot-level suppression study (Massachusetts), significant reduction in tipworm feeding injury inside the insecticide treated plots did not translate into greater fruit production in the following year. Werner and Platt (1976) hypothesized that perennial plants propagating by both sexual and vegetative means set an upper limit on the "biomass per stem" devoted to sexual reproduction and utilize the rest for "clonal expansion." Perennial plants also maintain a balance between reproductive and vegetative growth to optimize the available resources (Brown and McNeil 2006) and may explain results of the suppression study as well as that of no difference in fruit set among sites in the Massachusetts sampling study. However, the suppression study was limited in scale due to the short duration (one growing season) and relatively small size of the plots (as compared with the size of a typical commercial cranberry planting). These shortcomings preclude us from making broad generalizations regarding impact of tipworm injury suppression on harvestable crop and results of the study should be interpreted with caution. Trend from the sampling study indicates that the sites in Maine had higher proportions of tipworm-injured uprights as compared with the sites in Massachusetts. It is therefore possible that cranberry plantings in Maine could potentially benefit

relatively more (than plantings in Massachusetts) from either short and/or long-term injury suppression in terms of fruit production. Also, results from the Massachusetts suppression study are based on approximately 35% of the uprights with injury in the control plots (Howes), whereas the injury levels at two of the four Maine sites were in excess of 47% (Howes and Stevens). The impact of long term tipworm injury suppression on the fitness components of cranberry (sexual reproduction and clonal growth), and how it relates to commercial fruit production, remains yet to be determined in both Maine and Massachusetts.

In summary, the majority of individual cranberry uprights injured by tipworm larvae did not produce floral-units in the next growing season. We documented a trend of higher tipworm injury levels and lower flowering rates at the Maine sites, as compared with the sites in Massachusetts. However, the degree to which tipworm injury can negatively impact flowering/fruiting at the scale of a cranberry planting and the role played by differences in the environmental conditions (such as length of the growing season) between the two regions (Massachusetts and Maine) is yet to be determined. Our results thus partially support the hypothesis that tipworm will have a greater negative impact in the northern regions with a shorter growing season. Also, our plot-level suppression study (Massachusetts) did not find evidence of a negative relationship between tipworm injury and fruit output from one growing season to next. Thus, the proportion of uprights with tipworm injury at the end of growing season may be one of the factors that either independently, or through interactions with

environmental variables such as length of the growing season, impacts flowering and ultimately fruit production in commercial cranberry plantings.

CHAPTER 2

IMPACT OF EARLY SEASON APICAL MERISTEM INJURY BY GALL-INDUCING, *DASINEURA OXYCOCCANA* (DIPTERA: CECIDOMYIIDAE), ON REPRODUCTIVE AND VEGETATIVE GROWTH OF CRANBERRY

Introduction

In perennial woody plants, feeding by gall inducing insects can either have a negative impact on fitness (Dennill 1985, Sacchi et al. 1988, Martinez et al. 1992, DeClerck-Floate and Price 1994, Hayman et al. 2003, Dorchin and Adair 2011), or an indirect positive effect in the subsequent growing season (Nakamura and Ohgushi 2007). Some long-term studies have reported changes in the architecture and biomass of galled shoots but without an impact on fruit production (Kurzfeld-Zexer et al. 2010), a delayed negative effect on fruit production (Gonzales et al. 2005), or an immediate negative impact on fitness and a tendency to compensate over time (Sacchi and Connor 1999). Furthermore, the effects of herbivory in perennial woody plants may vary depending upon the level of physiological integration among the individual growth modules (e.g., shoots) (Haukioja and Koricheva 2000). Consequently, the damage at shoot level may not be representative of the effects at whole plant level and tolerance may be underestimated (Haukioja and Koricheva 2000).

The cranberry is a perennial clonal plant native to North America (Vander Kloet 1988) and commercial cultivation began ≈200 years ago in Massachusetts. Individual plants colonize the growing substrate through horizontal stolons

(runners), which produce vertical shoots called uprights (Fig. 7). Plants remain dormant during late fall and winter months (November-March) and resume growth in spring by either producing vegetative growth only (vegetative uprights), or vegetative growth combined with flowers in basipetal positions (flowering uprights) (Fig. 7). The flowers in cranberry uprights are borne along a vertical axis with sequential phenology; the earlier formed ones being closer to the base of current season growth (Birrenkott and Stang 1989, Brown and McNeil 2006) (Fig. 7). The number of flowers on an upright can range from 2-7 with 1-3 fruits reaching maturity by the end of the growing season (Brown and McNeil 2006). In addition to accruing vegetative growth annually, both flowering and vegetative uprights may produce additional uprights from buds at leaf axils (Eck 1990). The cultivars of cranberry include selections from native cranberry stands (e.g. Howes) as well as those developed through traditional breeding programs (e.g. Stevens and 'Crimson Queen'). Howes and Stevens are among the top three cultivars currently under cultivation in Massachusetts (acreage) and differ in terms of growth phenology and relative size of the fruit (Caruso 2008). The fruits in Stevens develop early and are relatively bigger as compared with the late maturing and small-fruited Howes. Cultivar Crimson Queen, a hybrid ('Ben Lear' × Stevens) released for commercial cultivation in 2006 from Rutgers University (Rutgers, NJ), is being planted by a number of growers in Massachusetts.

Cranberry tipworm is a multi-voltine gall-inducing fly whose larvae feed on apical meristem of cranberry uprights, with injury resulting in termination of growth. Tipworm larvae complete their development in ≈15 days and undergo three

instars (Gagne 1989). Pupation takes place in the uprights during the growing season whereas larvae of last generation fall to the ground for overwintering. In Massachusetts, tipworm adults emerge in mid-May and females oviposit on the growing uprights. Feeding injury to uprights by early generations of tipworm either precedes or coincides (depending on cultivar) with flowering and fruit set, a phenological stage when plants are under physiological stress from limited availability of carbohydrates (Birrenkott et al. 1991, Hagidimitriou and Roper 1994). In Massachusetts, the different life stages of tipworm can be detected on commercial cranberry plantings from May to September, although the peak period of activity coincides with early growth of uprights (early-June to early-July) (S. T., unpublished data). Similar seasonal dynamics of tipworm have been reported from Wisconsin (Voss 1996). Injured cranberry uprights may resume vegetative growth by producing side-shoots from lateral axillary buds in the current or subsequent growing seasons (Fig. 8). Tipworm is considered to be a pest in the cranberry growing regions of Canada, Maine, and northern Wisconsin (Mahr and Perry 2006, Cook et al. 2011, Charles Armstrong, personal communication). Tewari et al. (2012) reported that majority of uprights with tipworm feeding injury at the end of growing season do not produce flowers in the following year. However, the impact of early season tipworm feeding injury on reproductive and vegetative growth of different cranberry cultivars has not been quantified.

A two-year upright-level field study was carried out on Howes and Stevens at three locations to determine the impact of early season tipworm feeding injury and artificial meristem removal (simulated tipworm injury), on reproductive and

vegetative growth of flowering cranberry uprights. The following questions were addressed: 1) How does early season apical meristem injury (tipworm and artificial) impact fruit production of uprights in the current growing season? 2) What is the recovery potential of flowering uprights injured early in the season in terms of side-shoot production? 3) How does apical meristem injury impact vegetative growth and flower production of uprights in the next growing season? The first question was also investigated in a greenhouse study by comparing the response of three cultivars (Crimson Queen, Howes, and Stevens) to early season tipworm feeding injury.

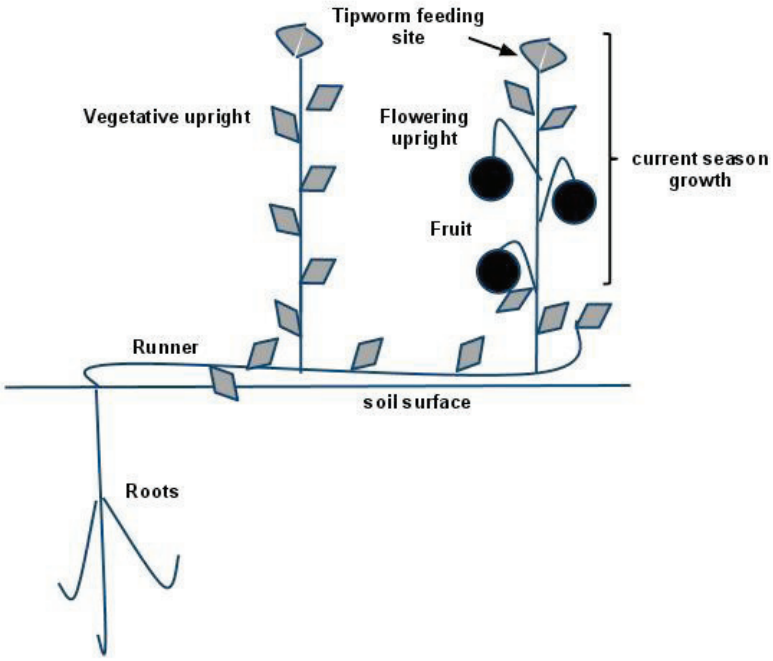

Figure 7. Cranberry plant showing a single runner (stolon) with flowering and vegetative uprights. Flowers and fruit develop in a basipetal position relative to current-season vegetative growth.

Figure 8. A cranberry upright showing tipworm feeding injury and side-shoot.

Materials and Methods

Upright – level field study (Year 1: 2010)

The study was carried out at three commercial cranberry production sites, two with Howes (HW1: 41°47'31.38" N 70°46'59.71" W, HW2: 41°48'08.98" N 70°44'04.27" W) and one with Stevens (ST1: 41°57'01.53" N 70°47'23.36" W). The plantings at sites HW1 and HW2 were ≈50 years old whereas site ST1 was planted in 1995. At each site, four 9 m transects equidistant from each other were marked in

a 0.2 ha plot. The transects were laid down perpendicular to the direction in which the majority of runners were oriented to eliminate measuring more than one upright on a single runner. Flowering uprights at the three sites were monitored for signs of tipworm feeding beginning mid-May and injury was detected (cupping of terminal leaves with dead apical meristem and characteristic scar tissue) in early June 2010.

In the first week of June 2010, 25 flowering uprights with visible tipworm feeding injury were tagged in each of the four transects at sites HW1 and HW2 using plastic slip-on tags printed with unique identification numbers (Texpak Inc., Franklin Square, NY). The tagged tipworm-injured uprights were approximately evenly spaced along the entire length of each transect. Two numbered series, each with 25 evenly spaced flowering uprights without tipworm feeding injury, were also tagged along each transect and one of the series was selected randomly to receive the simulated tipworm injury treatment (apical meristem was excised using a razor blade to mimic tipworm feeding injury). The remaining intact uprights (25) served as controls. A similar tagging and simulated injury treatment protocol was followed at site ST1. Twenty flowering uprights with visible tipworm feeding injury and 40 flowering uprights without injury were tagged along each transect. The simulated injury treatment was applied to half of the intact uprights (20 per transect) at site ST1 in the first week of June 2010. Thus, at HW1 and HW2, a total of 300 uprights were tagged (75 per transect × 4 transects) and at site ST1, 240 uprights were tagged (60 per transect × 4 transects). We measured the number of flowers per tagged upright at the beginning of study (June 2010) and the presence or absence of

side-shoots in September 2010. To ensure adequate pollination, honeybee hives were placed along the edge of the plantings at each site. Data on fruit yield of the tagged individual uprights were collected in September 2010 by harvesting all the fruits and measuring their fresh weight. Fruit set for each of the tagged uprights was calculated by dividing the number of mature fruits (diameter > 0.5 cm) on an upright in September 2010 by the total number of flowers present at the beginning of the study (June 2010).

Upright – level field study (Year 2: 2011)

35, 26, and 4 uprights tagged in 2010 at sites HW1, HW2, and ST1, respectively, could not be located in April 2011. All three sites used the flooding method of harvesting the tags may have been lost during the process. Intact, tipworm injured, and simulated injured uprights were approximately equally represented among the missing tagged uprights at each site. Data on new growth (present or absent) and flowering status (present or absent and number of flowers) from the remaining uprights were collected at the three sites in the first week of June 2011.

Greenhouse Study

We collected uprights of three cranberry cultivars (Crimson Queen, Howes, and Stevens) from established plots at the University of Massachusetts Cranberry Station (East Wareham, MA) in March 2011, before break of dormancy for the 2011-growing season. Collected uprights were planted in individual growing cells and

maintained under greenhouse conditions (University of Massachusetts Cranberry Station). In the last week of May 2011, for each cultivar, uprights with new growth and 4-5 unopened flowers were selected and divided into two equal groups. There were 50 total individual uprights for Howes and 60 each for Crimson Queen and Stevens. For each cultivar, one group was randomly assigned to the tipworm injury treatment and uprights from the other group served as controls. All the potted uprights were transported to a commercial planting with a history of tipworm infestation (41°56'54.35" N 70°47'31.50" W) and those in the injury treatment were placed among the vines on the cranberry bed. The control uprights were placed some distance away from the edge of the planting to prevent egg-deposition by tipworm females. All the uprights were watered daily and monitored for signs of tipworm feeding injury to the growing tip. Signs of tipworm feeding became visible after ≈3 weeks for the injury treatment uprights and all the plants were transported back to the greenhouse. In the greenhouse, control plants were monitored for signs of tipworm feeding injury to the growing tip. Similarly, plants in the injury treatment group were also checked to confirm disruption of apical growth due to tipworm feeding. Once the stigmas became exposed, all flowers were hand-pollinated using pollen collected from the same plots as the uprights for each cultivar (Sarracino and Vorsa 1991). All fruits having a diameter ≥ 0.5 cm at the beginning of September were counted to estimate fruit set. At the end of September 2011, fruits were harvested and fresh-weight was determined.

Data analysis

Upright – level field study (Year 1: 2010). We used SAS v. 9.3 for all the analyses (SAS Institute 2011). At each site, two-factor ANOVA (PROC GLM) was used to determine the impact of apical meristem injury (tipworm and simulated) and transect on fruit set and fruit weight. We also compared the number of flowers present per upright at the beginning of the study for the three types (tipworm injured, simulated injured, and intact). Both unopened and open flowers were included in the analysis. The Brown-Forsythe test was used to check the assumption for homogeneity of variance by combining transect and type of injury into one group for a total of 12 groups (3 injury types and 4 transects) per site. The Tukey-Kramer method was used to separate the least-square means for all significant tests.

We used a χ^2 based categorical data analysis model (PROC FREQ) with type of injury (natural vs. simulated) as the explanatory variable to determine if there was a difference in the production of side-shoots between uprights with either natural or simulated tipworm feeding injury. The impact of transect on the production of side-shoots was determined by comparing the two types of injured uprights (tipworm and simulated) across the four transects at each site. If no significant transect effects were detected, data on side-shoots were analyzed without a transect structure.

Data from 13, 37, and 20 uprights at sites HW1, HW2, and ST1, respectively, were not included in the analysis comparing fruit weight among the three types of tagged uprights (tipworm injured, simulated injured, and intact) because of fruit damage by larvae of *Acrobasis vaccinii* Riley (cranberry fruitworm), fruit rot

symptoms (caused by multiple agents) (Caruso 2008), or missing tags. All three types of uprights were represented approximately equally in the missing data.

Greenhouse study: We used two-factor ANOVA (PROC GLM) to determine if there was difference in fruit set among the three cultivars (Crimson Queen, Howes, and Stevens) and between tipworm injured and intact plants. The Brown-Forsythe test was used to check the assumption for homogeneity of variance by combining cultivars (three – Crimson Queen, Howes, and Stevens) and treatments (two – tipworm injured and intact) in one group for a total of six groups.

Data from five, five, and six control plants of Crimson Queen, Howes, and Stevens, respectively were not included in the analyses as a result of tipworm injury in the greenhouse. Similarly, data from two, one, and three plants of Crimson Queen, Howes, and Stevens, respectively, in tipworm injury treatment group, were not included in the analyses because feeding did not result in termination of apical growth. Fruits harvested from the majority of uprights in the greenhouse study (September 2011) showed symptoms of scald injury. This is caused by the interactions among abiotic factors such as temperature, amounts and duration of solar radiations, and humidity (Croft 2011). We therefore did not compare the effect of treatments on fruit weight.

Results

Upright – level field study (Year 1: 2010): Impact of early season apical meristem injury on number of flowers, fruit set, and fruit weight

Number of flowers. Injury type and transect did not have an effect on the number of flowers per upright and there was also no transect × injury type interaction at any of the three sites ($P > 0.05$ for all; Table 6).

Fruit set. There was a difference in fruit set among the three types of tagged uprights at site HW1 ($F = 3.52$; $df = 2, 287$; $P = 0.031$) and uprights with tipworm injury set more fruits than the intact uprights ($P = 0.027$, Tukey-Kramer adjusted). However, there was no difference in fruit set between tipworm-injured uprights and those with simulated injury at HW1 (Table 6). There was no difference in fruit set among the three types of uprights at sites HW2 and ST1 ($P > 0.05$). Furthermore, there was no transect effect or transect × injury type interaction at any of the three sites for fruit set (Table 6).

Fruit weight. There was difference in fruit weight among the three types of tagged uprights at the Howes sites (HW1: $F = 4.26$; $df = 2, 275$; $P = 0.0151$, HW2: $F = 4.07$; $df = 2, 248$; $P = 0.0182$). The weight of fruit from uprights with tipworm injury was higher than those from the intact uprights at HW1 ($P = 0.0169$, Tukey-Kramer adjusted). At HW2, weight of fruit from tipworm-injured uprights was higher than that of fruit from both intact uprights and those with simulated injury ($P = 0.02$; $P = 0.05$, Tukey-Kramer adjusted). There was also a significant transect effect for fruit weight at HW1 ($F = 5.83$; $df = 3, 275$; $P < 0.001$). The overall fruit weight in transect 4 (on plot edge) was higher than the two transects in the middle of plot ($P = 0.016$; P

< 0.001, both Tukey-Kramer adjusted). However, there was no transect × injury type interaction for fruit weight at HW1. Furthermore, there was no transect effect or transect × injury type interaction for fruit weight at HW2. At ST1, fruit weight did not differ among the three types of tagged uprights and there was neither a transect effect nor a transect × injury type interaction (Table 6).

Table 6. Mean number of flowers, proportion fruit set, and fruit weight in uprights that were either intact, tipworm injured, or with simulated injury at sites HW1, HW2, and ST1 in 2010 (L.S. Means).

Site	Type of Injury	Flowers at study start	Fruit Set	Fruit weight/upright (gm)
HW1	Intact	3.62 A[a]	0.55 B	1.92 B
	Tipworm	3.70 A	0.63 A	2.25 A
	Simulated	3.45 A	0.58 AB	2.0 AB
HW2	Intact	3.83 A	0.54 A	2.14 B
	Tipworm	4.16 A	0.58 A	2.47 A
	Simulated	3.97 A	0.53 A	2.10 B
ST1	Intact	4.58 A	0.36 A	2.74 A
	Tipworm	4.67 A	0.33 A	2.96 A
	Simulated	4.66 A	0.30 A	2.53 A

Production of side-shoots in response to early season tipworm and simulated apical meristem injury

At all sites (HW1, HW2, and ST1), only uprights with either tipworm or simulated injury produced side-shoots from lateral axillary buds (Fig. 9). At HW1, the production of side-shoots for uprights with tipworm injury varied among the four transects ($\chi^2 = 13.78$; df = 3; P = 0.003). Sixty-four percent of tipworm injured uprights in transect 1 produced side-shoot as compared with 21, 40, and 20% of the uprights in transects 2, 3, and 4, respectively. However, there was no transect effect for the production of side-shoots in uprights with simulated injury at HW1 (P > 0.05). The production of side-shoots for uprights with simulated injury (56%) was higher than the tipworm-injured uprights (21%) in transect 2 at HW1 ($\chi^2 = 6.37$; df = 1; P = 0.0115). There was no difference in production of side shoots between the two injury types in the remaining three transects at HW1 (P > 0.05). There was no transect effect in the production of side-shoots for both simulated and natural tipworm feeding injury at HW2 and ST1 (P > 0.05). In addition, there was no difference in the production of side-shoots between the two types of injury at HW2 and ST1 (P > 0.05).

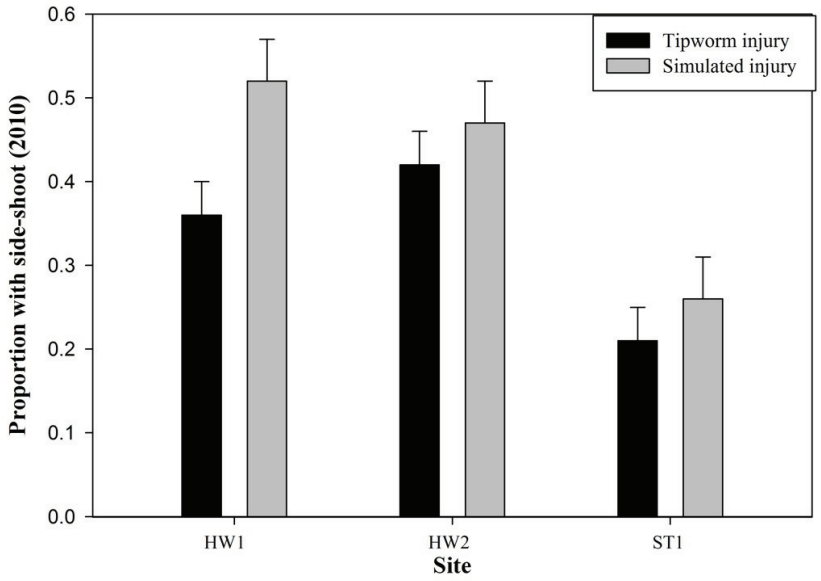

Figure 9. Proportion of uprights with side-shoots (mean, SE) after simulated and tipworm feeding injury at two Howes sites (HW1 and HW2), and a Stevens site (ST1). A significantly higher number of uprights with simulated injury produced side-shoots in transect 2 at HW1, but there was no difference between the two types of injury in the remaining three transects. There was no difference in production of side-shoots between the two types of injury at HW2 and ST1.

Upright-level field study (Year 2: 2011): Impact of apical meristem injury on vegetative growth and flowering in the next growing season

Vegetative growth – Overall. Fewer uprights with either type of injury (tipworm and simulated) resumed vegetative growth in 2011 as compared with the intact uprights at sites HW1 ($\chi^2 = 19.21$; df = 2; $P < 0.001$) and ST1 ($\chi^2 = 8.19$; df = 2; $P = 0.016$). The trend was similar at site HW2 ($P = 0.06$; Fig. 10).

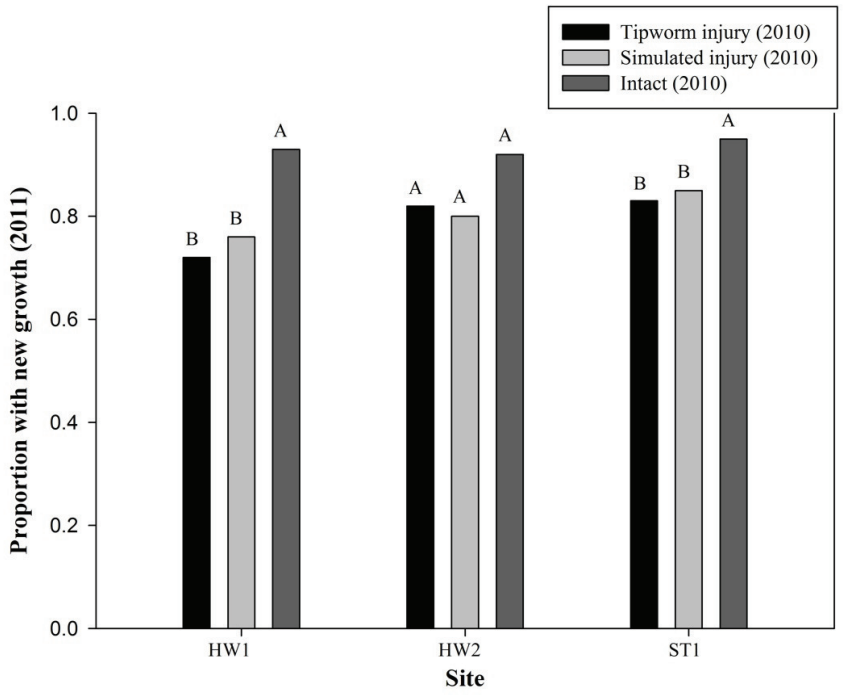

Figure 10. Proportion of uprights with new growth (2011) that were either intact, or with simulated and tipworm feeding injury in 2010 at sites HW1, HW2, and ST1. At each site, bars with the same letters above them are not significantly different (α = 0.05).

Vegetative growth - Uprights with side-shoots vs. uprights without side-shoots.

Significantly fewer uprights that lacked side-shoots resumed growth in 2011 as compared with the uprights that produced side-shoots for both types of injury at site HW1 (tipworm injury: $\chi^2 = 21.02$; df = 1; $P < 0.001$, simulated injury: $\chi^2 = 9.42$; df = 1; P = 0.002) and for tipworm-injured only at site ST1 ($\chi^2 = 17.35$; df = 1; $P < 0.001$; Table 7).

Table 7. Proportion of uprights with new growth (2011) that were tipworm injured (TI), or with simulated injury (SI) in 2010, and either produced side-shoots (with ss) or were without side-shoots (without ss), also in 2010.

Type of upright	HW1	HW2	ST1
TI (with ss)	0.93 A[a]	0.87 A	1.00 A
TI (without ss)	0.60 B	0.78 A	0.78 B
SI (with ss)	0.89 A	0.86 A	0.91 A
SI (without ss)	0.59 B	0.71 A	0.82 A

[a] Within each site and for the type of injury (tipworm and simulated), values followed by the same letters are not significantly different (Tukey-Kramer, $\alpha = 0.05$).

Flowering. Fewer uprights with either type of injury produced flowers in 2011 as compared with intact uprights at sites HW2 ($\chi^2 = 9.87$; df = 2; $P = 0.007$) and ST1 ($\chi^2 = 15.94$; df = 2; $P < 0.001$; Fig. 11). There was no difference in flowering among the three types of tagged uprights at site HW1 in 2011 ($P > 0.05$; Fig. 11). In addition, majority of uprights (> 96%) that did not produce side-shoots in 2010 also did not flower in 2011 for both simulated and tipworm injury at the three sites. The exceptions were 1, 3, and 4 uprights at sites HW1, HW2, and ST1, respectively; in which case, there was return flowering in the uprights that did not produce side-shoots in 2010.

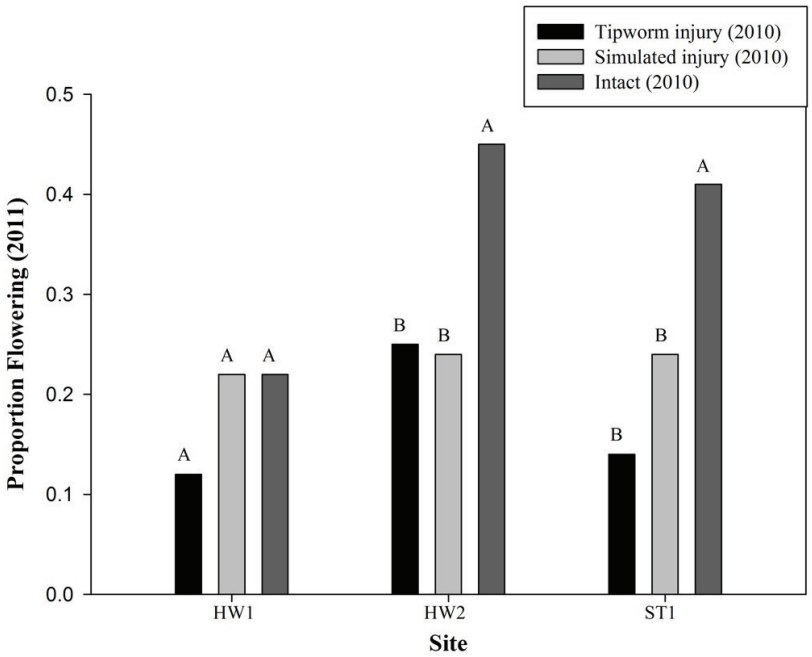

Figure 11. Proportion of uprights with flowers (2011) that were either intact, or with simulated and tipworm injury in 2010 at sites HW1, HW2, and ST1. At each site, bars with the same letters above them are not significantly different (α = 0.05).

Number of flowers per upright. The number of flowers produced per upright in 2011 did not differ between the intact uprights and those injured uprights that produced side-shoots in response to both simulated and tipworm injury (2010) (P > 0.05 for both) at the three sites (Table 8).

Table 8. Mean number of flowers (± SE) (2011) in uprights that were either intact, or with side-shoots (ss) after simulated (SI) or tipworm feeding injury (TI) in 2010.

Site/Type of upright	Intact	SI with ss	TI with ss
HW1	2.89 (0.32)	2.53 (0.28)	2.55 (0.34)
HW2	3.42 (0.20)	2.76 (0.31)	3.10 (0.29)
ST1	4.25 (0.21)	3.87 (0.41)	4.0 (0.33)

Greenhouse study

There was no difference in fruit set between the two treatments (tipworm-injured and control) and among the three cultivars ($P > 0.05$). There was also no cultivar × treatment interaction ($P > 0.05$). For Crimson Queen, Howes, and Stevens, the mean fruit set (± SE) per upright in the tipworm injury treatment was 0.36 (0.03), 0.39 (0.04), and 0.29 (0.03), respectively, and, in the control treatment was 0.27 (0.04), 0.33 (0.05), and 0.30 (0.05), respectively.

Discussion

The key finding of our study was that tipworm feeding injury does not affect the fruit output of cranberry uprights in the current growing season. However, the growth and flowering of the injured uprights was inhibited in the following year, a result that reinforces the importance of multiple-year studies for determining the impacts of herbivory in perennial plants (Strauss 1991, Karban and Strauss 1993,

Gonzales et al. 2005, Mueller et al. 2005; discussed in greater depth below). Gall inducing insects that feed on shoot meristems can impact fruit/seed production by either direct suppression of the flower buds (Dennill 1985, Fay and Hartnett 1991, Dorchin and Adair 2011) or via an indirect effect on the inflorescence (process of budding and blossoming) of galled shoots (Dennill 1985, Sacchi et al. 1988). In our field study, the number of flowers at the beginning of the growing season did not differ among the three types of uprights at all the sites. This result suggests that the termination of apical growth because of tipworm feeding and simulated injury occurred at a phenological stage when the formation of inflorescence was complete for Stevens, an early season cultivar, and also for the relatively late season Howes.

In the field and greenhouse, tipworm feeding and simulated injury to the flowering cranberry uprights did not reduce fruit set. In fact, fruit set in tipworm-injured uprights was higher than the intact uprights at site HW1. Actively growing apical meristems are a strong sink in plants and feeding injury by herbivores often results in their removal (Fay and Hartnett 1991). Additionally, injury to the apical meristems may also cause an alteration in the source-sink dynamics of plants (Utsumi and Ohgushi 2007). Overall, fruit set results from our field and greenhouse studies are in agreement with those reported in the literature for cranberry (Birrenkott and Stang 1989, Roper et al. 1992, Brown and McNeil 2006). Like fruit set, injury to apical meristem of uprights early in the growing season did not reduce fruit weight. Similarly, removal of new growth in spring did not affect fruit production in one-year-old branches of *Arctostaphylos uva-ursi* L. (bearberry), an ericaceous evergreen shrub (Salemaa et al. 1999).

The trend of higher fruit weight for the tipworm-injured uprights was consistent with that observed for fruit set at the three sites. In cranberry, fruit set is a key component of berry yield at the upright level (Eaton et al. 1983) and may explain the higher weight of fruit from tipworm-injured uprights as compared with intact ones at sites HW1 and HW2. The three potential sources of assimilates that support fruit development in cranberry are: i) current season growth of an upright, ii) one-year-old leaves on the same upright, and iii) leaves on adjacent uprights of the same runner (Roper and Klueh 1994). In addition, current season growth acropetal to the flowers/fruits has been identified as the main source of photosynthates for the developing fruit (Roper and Kleuh 1994, 1996). However, in our field study, disruption of acropetal growth early in the season (tipworm and simulated injury) did not reduce fruit weight and suggests that shifts in resource allocation patterns may take place following apical meristem injury. Physiological integration, resulting in the translocation of resources between individual ramets, has been reported in a number of perennial clonal plants (Newell 1982, Hutchings and Bradbury 1986, Watson 2008, Mony et al. 2011). In addition, events such as defoliation, shading, and herbivory promote sharing of resources between ramets (Ashmun et al. 1982, Hutchings and Bradbury 1986, Schmid et al. 1988, Alpert 1999, Wolfer and Straile 2012) and a similar phenomenon may explain the tolerance of apical meristem injury in cranberry. The relative size of fruit differs between the cultivars in cranberry. Howes, a small-fruited cultivar, produces more berries per unit area than the large fruited Stevens and yields are often similar (Roper 1991). This may explain the trend of similar fruit weight per upright for the two cultivars

despite fruit set being 40 – 50 % higher at the sites with Howes (HW1 and HW2), as compared with the site with Stevens (ST1) in the field study.

Many perennial plants respond to apical meristem injury by producing lateral shoots (Whitham and Mopper 1985, Marquis 1996, Wilson 2000, Nakamura and Ohgushi 2007). However, in our field study, the majority of flowering uprights with apical meristem injury (tipworm and simulated) did not resume vegetative growth (side-shoots) before the onset of dormancy (2010). Availability of meristems and allocation of sufficient resources are required for regrowth after herbivory (Bilbrough and Richards 1993, Marquis 1996). Vanden Heuvel and DeMoranville (2009) reported competition between the reproductive and vegetative growth in cranberry and a similar tradeoff might suppress the growth of side-shoots from lateral axillary buds in injured flowering uprights. However, it is also possible that re-growth response in cranberry is determined by meristem limitations (Whitham et al. 1991) as not all injured uprights may have active meristems to resume growth. In addition, we documented a trend of higher side-shoot production rate at the two Howes sites as compared with the Stevens site. Differences in age of the plantings (50 years at HW1 and HW2 vs. 15 years at ST1) may be one explanation for this observation. However, phenological differences between Howes (late maturing) and Stevens (early maturing) may have also contributed to the trend of higher side-shoot production at HW1, and HW2 as compared with ST1.

While the factors that influence re-growth after apical meristem injury are yet to be determined in cranberry, trends in flowering and growth of the tagged

uprights from the second year of study demonstrate the adaptive value of side-shoots. With few exceptions, only uprights with side-shoots produced flowers in the next growing season and the size of inflorescence (number of flowers) was comparable to that of the intact uprights. In cranberry, initiation of floral meristem for the next growing season begins in late June or early July of the current year (Eaton 1978) and results from our field study demonstrate that uprights that fail to produce side-shoots after injury do not initiate floral buds for the following year. In addition, fewer uprights that lacked side-shoots resumed growth in 2011 as compared with uprights with side-shoots for both types of injury at site HW1 and for tipworm injury only at site ST1. The trend in growth was similar for uprights with both types of injury (HW2) and simulated injury only (ST1) (Table 7). Similarly, galled shoots of *Salix lasiolepis* Bentham, a clonal perennial, experienced higher mortality and reduced growth in the years following infestation by *Euura lasiolepis* Smith (Craig et al. 1986). Feeding injury by the stem-boring larvae of *Dioryctria albovitella* Hust. caused mortality of terminal shoots in the pinyon pine, *Pinus edulis* Engelm. (Whitham and Mopper 1985). Martinez (2008) reported that galling of the axillary buds by *Slavum wertheimae* Hille Ris Lambers in *Pistacia atlantica* Desf. trees had a negative impact on the growth of apical buds in the next growing season. However, it is yet to be determined if the differences in new growth between the injured uprights (with and without side-shoots) in the second year of our field study was because of temporary cessation in growth or the result of upright mortality.

The impacts of upright level apical meristem injury were thus detected in the second year of the field study as fewer injured uprights resumed growth and produced flowers in 2011, as compared with the intact uprights (except at HW1 for flowering and HW2 for growth). In some perennial plants, the effects of herbivory become apparent over several growing seasons (Karban and Strauss 1993, Gonzales et al. 2005) and our study demonstrates that the vegetative and reproductive growth of cranberry uprights is suppressed in the year following tipworm-feeding injury. Furthermore, it is possible that some of the growth parameters not measured in our study (clonal expansion, below-ground biomass and/or storage of resources, etc.) are also adversely impacted by tipworm injury and that the negative effects are manifested over an extended temporal scale (> 2 years). In woody perennials, the cumulative effect of multi-year herbivory can be significant (Whitham and Mopper 1985, Strauss 1991, Mueller et al. 2005) and may result in altered branch architecture, reduced growth rate, and decline in reproductive output (Whitham and Mopper 1985). A number of cranberry production sites in Massachusetts, especially those with Howes, experience high levels of recurring annual tipworm infestation (S. Tewari, unpublished data). However, the impacts of chronic multiple year tipworm feeding injury on the growth and reproduction of cranberry are yet to be determined. Furthermore, physiological processes such as return bloom and return fruit vary among the growing regions of cranberry (Strik et al. 1991). It is therefore possible that the inter-annual inhibition of upright growth and flowering because of tipworm injury may be exacerbated by the shorter length of growing

season in the cranberry production regions of northern latitudes (Canada, Maine, northern Wisconsin; tipworm-injury × environment interaction).

Although we documented reduced return flowering for uprights with injury at two of the three field sites in 2011, our study does not address whether this may translate into diminished fruit yield at the clonal or planting level in mature cranberry beds (as opposed to individual uprights). Haukioja and Koricheva (2000) cautioned that short-term studies done at the shoot level in woody plants might underestimate tolerance to herbivory because the effect of injury on individual modules (uprights in our study) may be different than the response at the whole-plant level. At the whole-plant or clonal level, a mature cranberry planting consists of an interconnected population of vegetative and flowering uprights or ramets, the majority being vegetative. It is therefore possible that effects of injury-related inhibition of flowering and growth of individual uprights, as detected in the second year of our field study, might be offset by compensatory reproductive and vegetative growth in injury-free uprights of the same plant. Briske and Butler (1989) reported that ramet recruitment within a clone increased in response to thinning of ramets in *Schizachyrium scoparium* var. *frequens* Hubb., a perennial clonal plant. Similarly, despite changes in births and deaths of ramets during a growing season, the overall population of ramets at the end of the year was similar to that recorded at the beginning of the season in two different growing environments for *Ranunculus repens* L., also a perennial clonal (Doust 1981). Thus, in a mature cranberry planting, the overall effect of injury from one growing season to the next may be negligible at the whole plant level and not warrant management strategies against tipworm in

Massachusetts. A replicated short-term (two-year) insecticide exclusion study done at a commercial Howes planting in Massachusetts suggests that this may be the case; significant reduction in tipworm injury at the plot level (3.81 m^2) did not increase flower and fruit production in the next growing season (Tewari et al. 2012). Insecticide based exclusion studies have been utilized to determine the impacts of herbivory at whole plant level (Whitham and Mopper 1985). We suggest that multiple-year exclusion studies carried out on mature plantings of different cranberry cultivars may be required to determine the impacts of chronic tipworm injury at clonal or whole plant level.

CHAPTER 3

PHYSIOLOGICAL INTEGRATION PLAYS KEY ROLE IN CRANBERRY TOLERANCE OF DAMAGE BY TIPWORM LARVAE, *DASINEURA OXYCOCCANA* (DIPTERA: CECIDOMYIIDAE)

Introduction

Plants are composed of functional units called metameres, each consisting of a meristem, node, internode, and one or more leaves (Watson 1986, Marquis 1996). Individual or multiple metameres can act as a morphological unit with respect to the movement of resources (especially carbon), with the implication that resources move easily within a unit as compared to between units (Marquis 1996). Thus, distinct physiological subunits can be identified based on the assimilation, distribution, and utilization of carbon in plants (Watson and Casper 1984). Integration between these units and resource allocation patterns within plants play an important role in determining the tolerance response to herbivory (Trumble et al. 1993, Marquis 1996). Physiological integration between connected shoots has been reported in clonal plants and is dependent upon the presence of sinks or actively growing tissues (Newell 1982, Hutchings and Bradbury 1986, Landa et al. 1992, Svensson 1995). In addition, a number of studies on perennial clonal plants have demonstrated that events such as shading, defoliation, and herbivory result in compensatory movement of resources to the disturbed modules (Ashmun et al. 1982, Hartnett and Bazzaz 1983, Hutchings and Bradbury 1986, Schmid et al. 1988, Alpert 1999, Zhang et al. 2002, Wolfer and Straile 2012). The shifts in resource allocation patterns can be viewed as adaptive responses to unfavorable conditions

(Ashmun et al. 1982, Hartnett and Bazzaz 1983) and may play a role in the tolerance of herbivory in clonal plants.

The cultivated cranberry (*Vaccinium macrocarpon* Aiton) is a woody, low-growing perennial that colonizes the soil surface through horizontal runners (stolons). Vertical shoots called uprights are borne along the runners at regular intervals. Each growing season, the uprights either produce vegetative growth only (vegetative uprights), or vegetative growth with flowers/fruits in the basipetal positions (flowering uprights) (Fig. 12). Like other clonal plants, the runners of cranberry can send out adventitious roots from the leaf axils under suitable conditions (Eck 1990). Cultivars of cranberry consist of selections from wild native stands (e.g. Howes), and those developed through conventional breeding programs (e.g. Stevens). The berry crop in Howes is late maturing and small-fruited as compared to the early maturing and large-fruited Stevens. Cranberry tipworm is a multivoltine gall making fly that overwinters as late instar larvae (Gagne 1989) and adults emerge around mid-May in Massachusetts. Tipworm larvae feed on actively growing apical meristematic tissue of the uprights (Cook et al. 2011) and injury results in the termination of vegetative growth. There can be multiple overlapping generations of cranberry tipworm in a single growing season (Gagne 1989).

In the cultivated cranberry, current season growth of an upright (Fig. 12) has been identified as the main source of assimilates for the developing fruits (Roper and Kleuh 1994, 1996). The two other sources that can also contribute assimilates fixed in the current growing season are: i) one year old leaves on the same upright, and ii) leaves on adjacent uprights along the same runner (Roper and Kleuh 1994)

(Fig. 12). Although feeding by tipworm larvae early in the growing season (mid May – mid June) disrupts growth, individual uprights of cranberry can tolerate the injury without a negative impact on fruit output (S. T., unpublished data). However, the relative contribution of different assimilate sources to the developing fruits in tipworm-injured uprights has not been determined. Quantifying the contribution of current season growth to fruit output will suggest potential tolerance mechanisms (e.g., physiological integration) in tipworm-injured uprights. Furthermore, understanding the mechanism will be a key step in determining any costs that may be associated with tolerance of apical meristem injury in perennial cranberry.

The goal of this study was to determine if physiological integration among the different sources of assimilates for the developing fruits in cranberry plays a role in the tolerance of tipworm feeding injury. We quantified the contribution of current season acropetal growth to the fruits by girdling tipworm-injured flowering uprights (Fig. 13). This technique enabled us to disrupt the flow of assimilates to and from the current season growth (including developing fruits) for the entire growing season (as opposed to the short-term studies demonstrating physiological integration by monitoring the movement of radioactive carbon isotopes among the morphological subunits). The following question was addressed: Is physiological integration between the current season growth and other potential sources of assimilate important for fruit growth in tipworm-injured uprights? The question was investigated in two cranberry cultivars (Howes and Stevens).

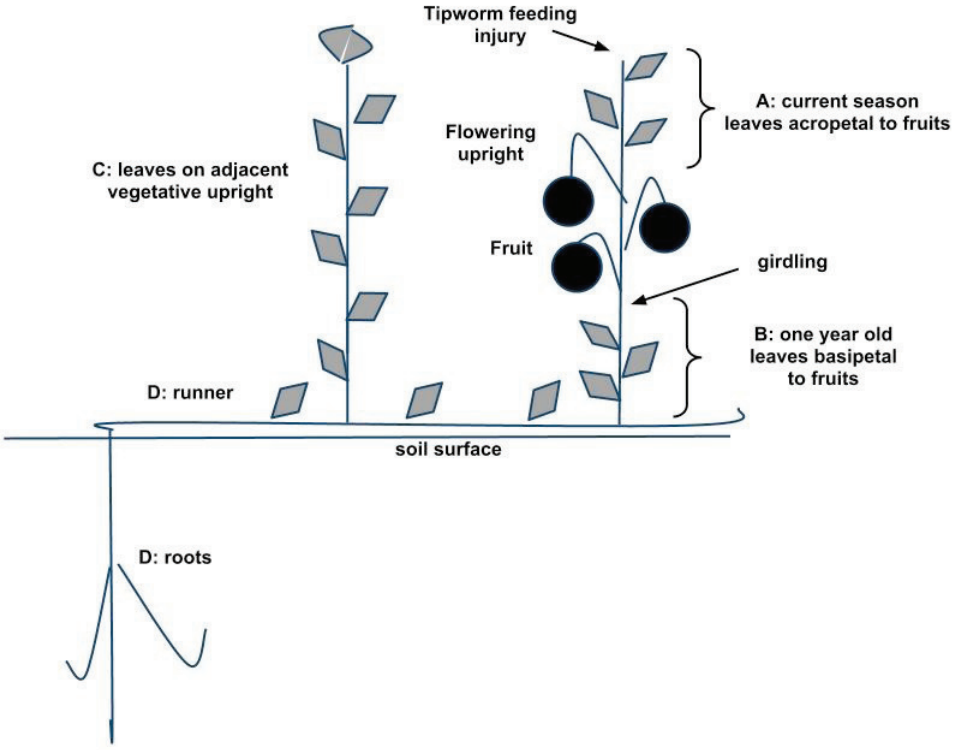

Figure 12. A section of cranberry vine showing the different assimilate sources for developing fruits on a flowering upright. A) leaves on current season growth acropetal to fruits, B) one year old leaves, C) leaves on adjacent vegetative uprights on the same runner, D) stored reserves in the roots and runners.

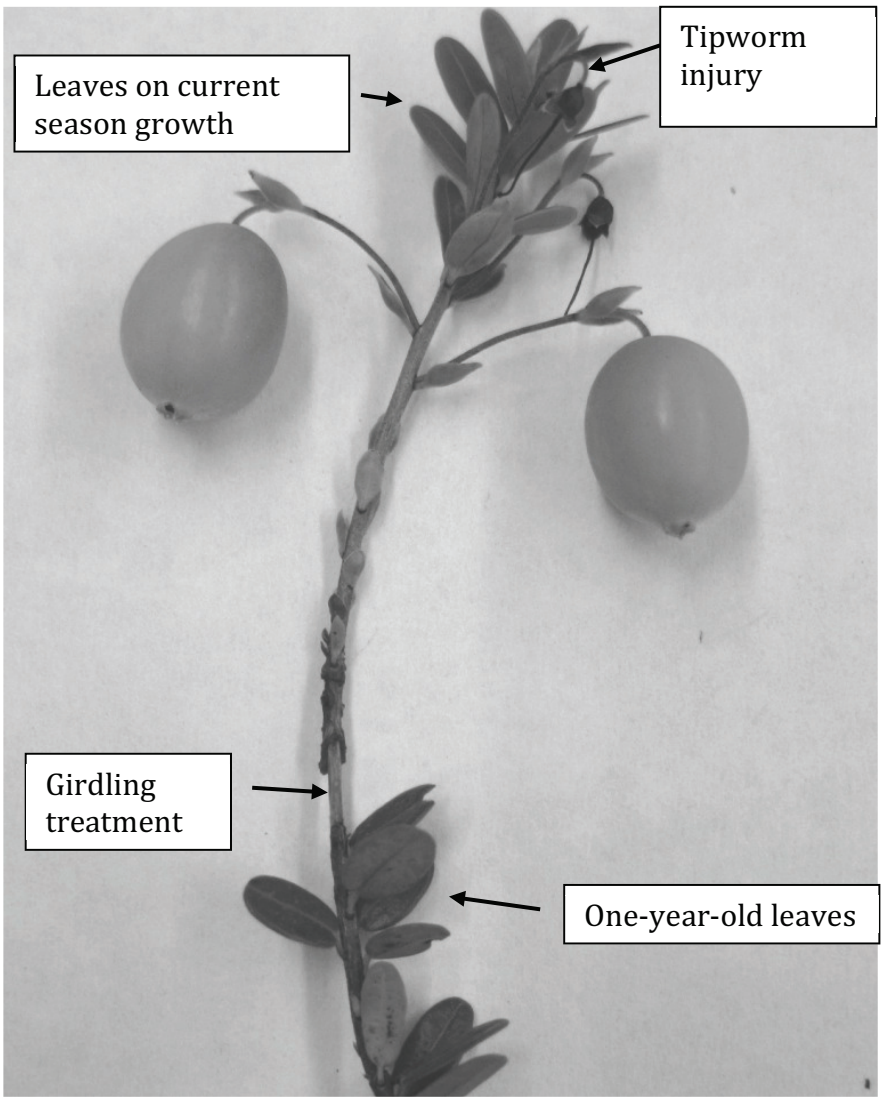

Figure 13. A tipworm-injured flowering cranberry upright with girdling treatment applied at the base of current season growth. Also visible are one-year-old leaves and leaves on the current season growth.

Materials and methods

Determining the role of current season growth in tolerance of apical meristem injury (Field Study)

The study was carried out at a commercial cranberry farm with established beds of Howes and Stevens (41°56'59.31" N 70°47'15.98" W), planted in 1985 and 1995, respectively. For each cultivar, one 0.2 ha plot was established and four 10 m equidistant transects were marked in each cultivar plot. The transects were laid down perpendicular to the direction in which the majority of runners was oriented to eliminate measuring more than one upright on a single runner. For each transect in both the cultivar plots, 30 flowering uprights with visible tipworm feeding injury signs were identified (cupping of terminal leaves with dead apical meristem and characteristic scar tissue) and tagged in the first week of June 2011 using plastic slip-on tags printed with unique identification numbers (Texpak Inc., Franklin Square, NY). Fifteen intact flowering uprights without injury were also tagged along each transect. A total of 180 individual flowering uprights were tagged for each cultivar (45 per transect × 4 transects). The tagged tipworm-injured and intact uprights were approximately evenly spaced along the entire length of each transect. For each transect in both the cultivar plots, half of the tipworm-injured uprights were chosen randomly to receive a girdling treatment in the second week of June 2011. The outer bark tissue (including phloem) of the upright was removed using a razor blade, just below the point where growth for the 2011 season was initiated, and the xylem was left intact (Fig. 13). The goal was to prevent the movement of assimilates either to or from the current season growth, including

developing fruits, of the tagged uprights. At the end of September 2011, fruits were harvested from the tagged uprights and fresh weight was determined. Fruit set for each of the tagged uprights was calculated by dividing the number of mature fruits on an upright (fresh weight > 0.4 g) by the total number of flowers present at the beginning of the study (June 2011). After fruit removal, uprights with tipworm injury (girdled and non-girdled) were harvested and area of all the leaves on the current season's growth was measured using a leaf area meter (LI-COR Biosciences, Lincoln, NE). The sample of leaves from each upright was run through the leaf area meter three times and the mean value was used in statistical analysis.

Effect of girdling on growth and movement of assimilates in intact cranberry uprights (supplement to field study)

A separate study was carried out to: 1) detect the effects of injury sustained during girdling on the reproductive and vegetative growth of intact cranberry uprights, and 2) determine the effectiveness of the girdling technique in disrupting the flow of assimilates in intact cranberry uprights. Sixty-six individual undamaged uprights were tagged in May 2011 using plastic slip-on tags in a 12 × 24 m plot of cultivar Howes, planted in 2007 at the University of Massachusetts Cranberry Station (East Wareham, MA) (41°46'02.00" N 70°40'09.53" W). One of the following treatments was applied randomly to 22 of the tagged uprights on May 15, 2011: i) girdling only, ii) girdling and defoliation, and iii) control (Fig. 14). The terminal buds of the tagged uprights had begun to swell but were still not open at the time of treatment application (Fig. 14). Girdling was done approximately 2 cm below the growth initiation point for the 2011 season, and all the one-year-old leaves above it

were removed in the defoliation treatment (Fig. 14). The following responses were measured: i) length of current season growth, ii) flowering status of the upright, iii) number of flowers, iv) fruit set, and v) fruit weight. To compare the length of new growth on the uprights among the three treatments, data collected on August 4, 2011 were used in the statistical analysis. Fruit set for each of the tagged uprights was calculated by dividing the number of mature fruits on an upright (fresh weight > 0.4 g) by the total number of flowers present at the beginning of the study in June 2011. Fruits were harvested from all the uprights at the beginning of September 2011 and fresh weight was measured.

The effect of girdling on the movement of assimilates can thus be indirectly estimated by comparing the intact uprights and those that received the girdling and defoliation treatment for the responses measured. Similarly, the effect of girdling on the growth of uprights can be detected by comparing the intact uprights with those that received the girdling treatment only.

Figure 14. Uprights without tipworm feeding injury showing the three treatments used to determine the impact of girdling and defoliation on reproductive and vegetative growth of cranberry (supplement to field study). A) control, B) girdling only, and C) girdling and defoliation.

Data Analysis

Field Study. We used SAS v. 9.3 for all the analyses (SAS Institute 2011) and separate analyses were carried out for the two cultivars (Howes and Stevens). Weighted two-factor ANOVA (PROC GLM) was used to compare the weight of fruit from tipworm injured (girdled), tipworm-injured (non-girdled), and intact uprights. For the 12 treatment – transect groups; weight is defined as the inverse of variance for each group. Transect was included as a fixed factor and the Tukey-Kramer method was used to separate the least-square means. We also compared leaf area of

the current season growth between girdled and non-girdled tipworm-injured uprights. The direction and strength of relationship between current season leaf area and fruit weight in the girdled and non-girdled tipworm-injured uprights was determined using separate correlation analyses (PROC CORR). These analyses were carried out to provide indirect evidence of whether the girdling technique was effective in disrupting the flow of assimilates.

One of the transects in cultivar Howes was found to be in a section of the plot where the majority of fruits exhibited symptoms of fruit rot caused by multiple agents (Caruso 2008). Therefore, data from the uprights tagged in this transect were excluded from analyses.

Supplement to field study. One-way ANOVA (PROC GLM) was used to determine the impact of the girdling and defoliation treatments on vegetative and reproductive growth of cranberry uprights. The Tukey-Kramer method was used to separate the least-square means. A χ^2 based categorical data analysis test (PROC FREQ), with treatment as the explanatory variable, was used to determine if there was difference in the growth habit (flowering or vegetative) of the uprights that received the three treatments (girdling only, girdling and defoliation, and control).

Only two of the 22 uprights in the treatment that included both girdling and defoliation produced flowers and none of the uprights in that treatment set fruit. Therefore, we compared uprights that received girdling only treatment with the control uprights for flower number, fruit set, and fruit weight.

Results

Determining the role of physiological integration in the tolerance of tipworm feeding injury

Fruit weight. The fruit weight differed between tipworm-injured (girdled), tipworm-injured (non-girdled), and intact uprights for both cultivars (Howes: $F = 73.19$; $df = 2, 123$; $P < 0.0001$, Stevens: $F = 74.28$; $df = 2, 161$; $P < 0.0001$; Fig. 15). Fruit weight from girdled uprights with tipworm injury was lower than non-girdled tipworm-injured uprights (Howes: $P < 0.0001$, Stevens: $P < 0.0001$), and also the intact uprights (Howes: $P < 0.0001$; Stevens: $P < 0.0001$). Fruit weight did not differ between the intact uprights and tipworm-injured non-girdled uprights for both Howes and Stevens ($P > 0.05$). There was a transect effect for both cultivars (Howes: $F = 7.12$; $df = 2, 123$; $P = 0.0012$, Stevens: $F = 6.79$; $df = 3, 161$; $P = 0.0002$) and also a significant treatment × transect interaction (Howes: $F = 3.02$; $df = 4, 123$; $P = 0.0204$, Stevens: $F = 2.59$; $df = 6, 161$; $P = 0.0202$).

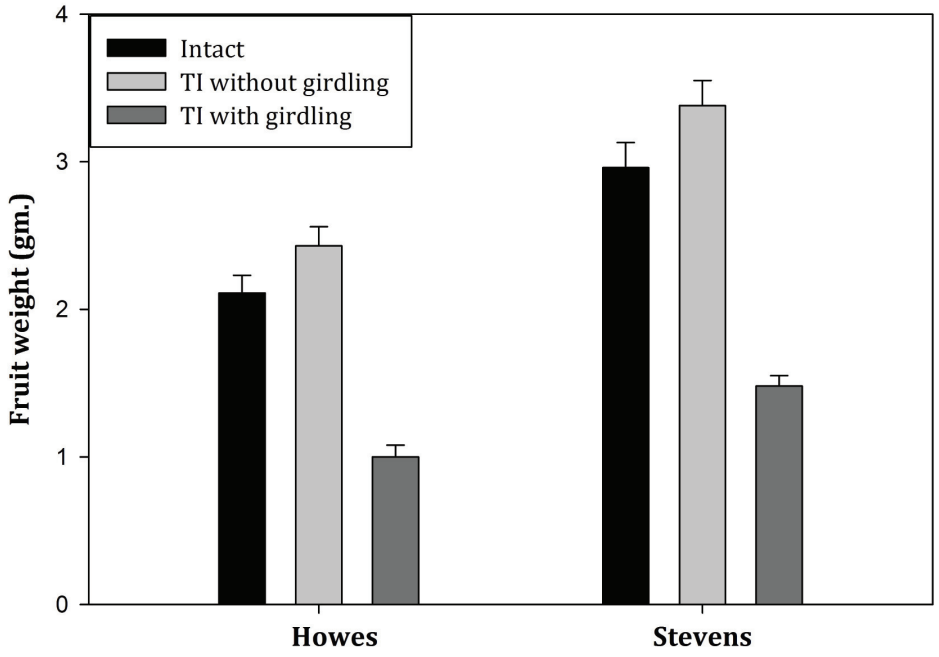

Figure 15. Fruit weight (g) in uprights that were either intact, or tipworm injured (TI) without girdling, or tipworm injured with girdling (mean ± SE).

Current season leaf area in girdled vs. non-girdled tipworm-injured uprights. There was no difference in current season leaf area (cm^2) between the girdled and non-girdled tipworm-injured uprights for both cultivars (Howes: girdled: 2.53 (±0.15), non-girdled: 2.33 (±0.15); Stevens: girdled: 2.70 (±0.13), non-girdled: 2.90 (±0.11)). There was a significant and positive correlation between fruit weight and current season leaf area in girdled uprights of both cultivars (Howes: $r = 0.72$, $N = 42$, $P < 0.0001$; Stevens: $r = 0.79$, $N = 54$, $P < 0.0001$; Figs. 16 and 17). However, the relationship was not significant for the non-girdled uprights in both the cultivars ($P > 0.05$).

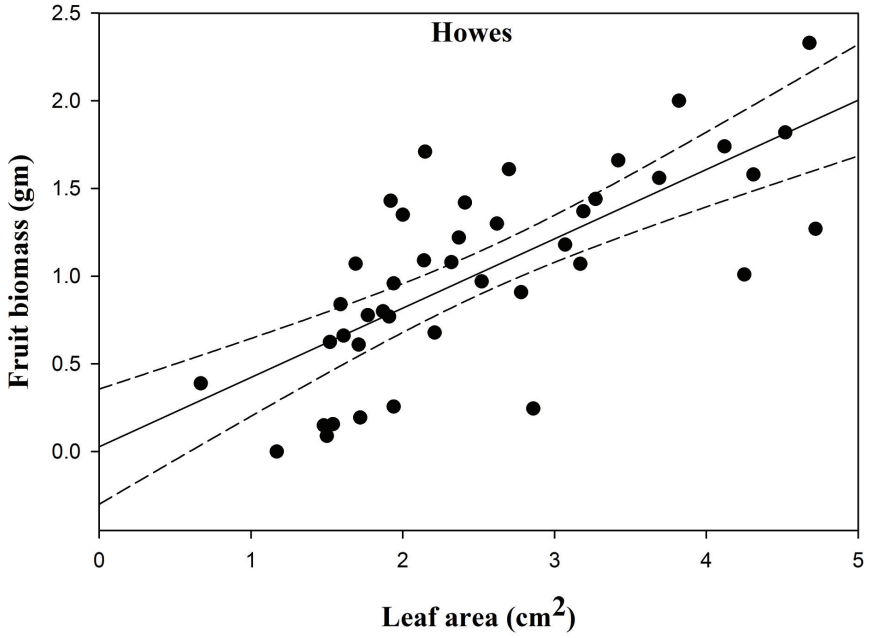

Figure 16. First order regression line (with 95% CI) showing significant positive relationship between fruit weight and leaf area of current season growth in tipworm-injured girdled uprights of Howes.

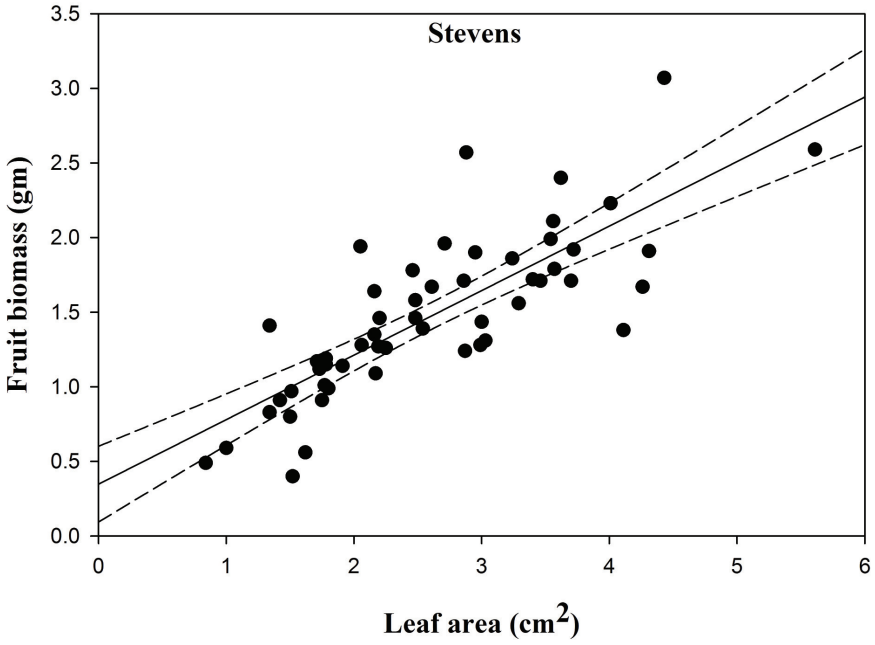

Figure 17. First order regression line (with 95% CI) showing significant positive relationship between fruit weight and leaf area of current season growth in tipworm-injured girdled uprights of Stevens.

Effect of girdling on growth and movement of assimilates in intact cranberry uprights

Treatments affected length of the current season growth ($F = 41.75$; $df = 2, 62$; $P < 0.0001$; Table 9). Control uprights and those that received only the girdling treatment grew longer than uprights that were both girdled and defoliated ($P < 0.0001$ for both comparisons). Also, in 10 of the 22 uprights that were both girdled and defoliated, the current growth was shriveled and appeared dead in September 2011. There was no difference in the length of current season growth between the control uprights and those with girdling treatment only ($P > 0.05$). Treatment also

affected growth habit (flowering vs. vegetative) (χ^2 = 49.22; df = 2; P < 0.0001). Only two of the 22 uprights in the treatment with both the girdling and defoliation produced flowers, compared to 21 of the 22 uprights in the control and the girdling only treatments. There was no difference between the control uprights and those with girdling only treatment for the number of flowers, fruit set, and fruit weight (P > 0.05; Table 9).

Table 9. The impact of girdling (G) and defoliation (DF) on the vegetative and reproductive growth variables of intact cranberry uprights (all L.S. Means).

Variable/Treatment	Intact	Intact with G	Intact with G+DF
Current season growth (cm)	4.62 A[1]	4.26 A	2.24 B
Number of flowers	3.45 A	3.57 A	-[2]
Fruit set	0.57 A	0.53 A	-[2]
Fruit weight (g)	1.68 A	1.15 A	-[2]

[1] For each variable, values followed by the same letter are not significantly different (Tukey-Kramer, α = 0.05).

[2] Only two uprights out of the 22 produced flowers and none set fruit in the G+DF group.

Discussion

The key finding of our study was that in cranberry, physiological integration between the current season growth and other sources of assimilates plays a

significant role in the tolerance of tipworm feeding injury. Mean fruit weight declined by more than 55% in the girdled tipworm-injured uprights as compared to the non-girdled uprights (Howes and Stevens); girdling depleted assimilates available from sources other than the current season growth (one year old leaves on the same upright, adjacent uprights, runners, and/or stored reserves).

Developing fruits can be a strong sink and thus influence the partitioning of assimilates at plant level (Cannell 1971, Schaffer et al. 1986, Ho 1988). Furthermore, the movement of assimilates in clonal plants may take place within and among the morphological subunits or ramets (Newell 1982, Svensson 1995, Watson 2008, Mony et al. 2011). Karlsson (1994) reported that one-year-old leaves were an important source of carbon for current year reproduction in *Rhododendron lapponicum* (L.) Wahlenb, an evergreen ericaceous clonal shrub. Movement of resources between ramets of clonal plants, specifically in response to events like defoliation, shading, and herbivory has also been reported (Ashmun et al. 1982, Hartnett and Bazzaz 1983, Hutchings and Bradbury 1986, Schmid et al. 1988, Alpert 1999, Zhang et al. 2002, Wolfer and Straile 2012). Similarly, our study demonstrates that developing fruits in tipworm-injured uprights receive assimilates from other parts of the plant.

The phenological stage plays an important role in determining the priority of assimilate partitioning in plants (Ho 1988). Roper and Kuleh (1996) labeled uprights with $^{14}CO_2$ to determine the carbon source for the developing flowers and fruits in cranberry. Current season growth acropetal to the floral structures was found to be the major contributor of carbon during both flowering

and fruiting stages (Fig. 12). In addition, almost no activity was found to move from either one-year-old leaves on the same upright, or from those on the adjacent vegetative uprights to the flowers/fruits (Fig. 12). However, results from our study suggest that following tipworm feeding injury to the flowering uprights, resource allocation patterns may undergo modifications to meet the assimilate demand of the developing fruits. The $^{14}CO_2$ labeling experiment (Roper and Kuleh 1996) was a short duration study done at two specific phenological stages of annual cranberry growth cycle (flowering and fruiting). In contrast, we disrupted the flow of assimilates for the entire growing season. This may explain the discrepancy in results between our study and that of Roper and Kuleh (1996), and suggests that longer-term studies may provide important insights into resource allocation patterns that drive responses to damage.

Stored resources play an integral role in the expression of life history traits in perennial clonal plants (Watson 2008). Watson (2008) hypothesized that in clonal plants, carbon fixed in the current year does not usually support or rescue growth and reproduction. These processes are instead dependent on the resources accumulated in the previous years (Watson 2008). Furthermore, because biennial bearing has been reported in cranberry (Roper et al. 1993), stored resources can be expected to play an important role in flowering and fruiting (Crawley 1983). It is yet to be determined if the assimilates supplied to the developing fruits in tipworm-injured uprights are fixed in the current growing season, or mobilized from resources stored in the current or previous years (Landa et al. 1992, Watson 2008). Future experiments using radioactive isotopes may provide more information on

the relative contribution of the different assimilate sources to tolerance of tipworm injury in cranberry. If present, the determination of a link between stored resources and the response to tipworm feeding injury will help frame questions related to potential costs associated with the tolerance documented in our study. The possible link between stored resources and tolerance of tipworm-injury may also help to explain the apparent discrepancy between the results of our study and those reported by Roper and Kuleh (1996).

There was no difference in the current season leaf area between the girdled and non-girdled tipworm-injured uprights. Furthermore, fruit weight was positively correlated to the leaf area in the girdled uprights only for both cultivars. These results, combined with those from the supplement study, confirm that our girdling technique was successful in disrupting the movement of assimilates. The combined effect of girdling and defoliation in the supplement study was detrimental to all measured growth parameters, and approximately half of the uprights appeared dead in September 2011. However, girdling alone did not impact the vegetative and reproductive growth of intact uprights compared to control uprights, suggesting that flowering uprights are able to tolerate the injury associated with the removal of bark tissue.

Majority of cranberry acreage in North America consists of Stevens (Roper 2001) whereas Howes is planted in approximately 30% of Massachusetts's commercial bogs. The two cultivars differ in terms of phenology, fruit set, and the size of individual fruits. Stevens, a phenologically advanced cultivar, produces fewer large fruits per upright as compared to the late season and small-fruited Howes.

Girdling of the tipworm-injured flowering uprights resulted in an approximately equal fruit weight decline for both cultivars (59% in Howes and 56% in Stevens). This result suggests that following tipworm feeding injury, similar resource allocation patterns exist in the two cultivars and comparable trends may be expected from the other cranberry cultivars in North America.

In summary, our study demonstrated that integration between the different sources of assimilates is important for the tolerance of tipworm feeding injury in cranberry. However, it is yet to be determined if the resources mobilized to the fruits in tipworm-injured uprights are fixed in the current growing season, or whether stored reserves supply the majority of assimilates. A number of commercial cranberry plantings in Massachusetts, especially those with Howes, are subject to high levels of recurring annual tipworm infestations (S. Tewari unpublished data). We suggest that long-term studies in which tipworm densities are experimentally manipulated over a range, or are suppressed for multiple growing seasons, may shed more light on the nature of tolerance exhibited by flowering uprights of cranberry.

CHAPTER 4

DEVELOPING FRUITS INHIBIT THE REGROWTH OF CRANBERRY SHOOTS AFTER APICAL MERISTEM INJURY BY LARVAE OF *DASINEURA OXYCOCCANA* (DIPTERA: CECIDOMYIIDAE).

Introduction

Vegetative growth is an important fitness component in perennials, especially for plants that also reproduce clonally (Stevens et al. 2008). Following apical meristem injury, vegetative regrowth from the undamaged lateral meristems is made possible by the metameric architecture of plants (Marquis 1996). Production of new lateral shoots by the activation of dormant buds is a common tolerance mechanism against apical meristem herbivory (Whitham and Mopper 1985, Tolvanen et al. 1994, Marquis 1996, Lehtila 2000, Wilson 2000, Nakamura and Ohgushi 2007). However, regrowth after injury may be constrained by factors such as meristematic limitations (Richards and Cladwell 1985), and the allocation of sufficient resources to the dormant buds (Bilbrough and Richards 1993).

The cultivated cranberry, *Vaccinium macrocarpon* Aiton, is a low-growing woody perennial native to North America (Vander Kloet 1988). Horizontal stolons, also know as runners, trail on the soil surface and produce vertical shoots called uprights. Each year the uprights may either produce vegetative growth only (vegetative uprights; Fig. 18), or vegetative growth combined with flowers/fruits in basipetal positions (flowering upright; Fig. 19). Cultivars of cranberry are either selections from wild native stands (e.g. Ben Lear and Howes), or those developed through traditional breeding programs (e.g. Stevens). Cranberry is commercially

cultivated in Massachusetts, New Jersey, Oregon, Washington, Wisconsin, and Maine. In Canada, the major cranberry production regions are British Columbia and Quebec (Agriculture and Agri-Food Canada 2009). Tipworm is a gall making fly that overwinters as late instar larvae (Gagne 1989) and adults emerge around mid-May in Massachusetts. Tipworm females lay one or more eggs on the actively growing cranberry uprights and the larvae kill the tip by piercing through the meristematic tissue (Cook et al. 2011; Figs. 18 and 19). The life cycle from eggs to adults takes 10-15 d (Mahr 1996) and there can be multiple overlapping generations of tipworm in a single growing season (Gagne 1989).

After tipworm feeding injury, cranberry uprights may resume vegetative growth by the activation of lateral meristems and form a side-shoot (Figs. 18 and 19). However, the majority of tipworm-injured flowering uprights do not produce a side-shoot before the onset of dormancy and growth (vegetative and reproductive) in the following year may be adversely affected (S Tewari unpublished data). It is yet to be determined if lack of active meristems (Whitham et al. 1991) prevents regrowth after tipworm feeding injury, or whether presence of developing fruits inhibits the production of side-shoots through sink-sink competition (Ho 1988) in flowering cranberry uprights. Our study addresses the following questions: 1) Is there a difference between tipworm-injured vegetative and flowering uprights in the production of side-shoots? and 2) Does the presence of developing fruits suppress regrowth after tipworm feeding injury?

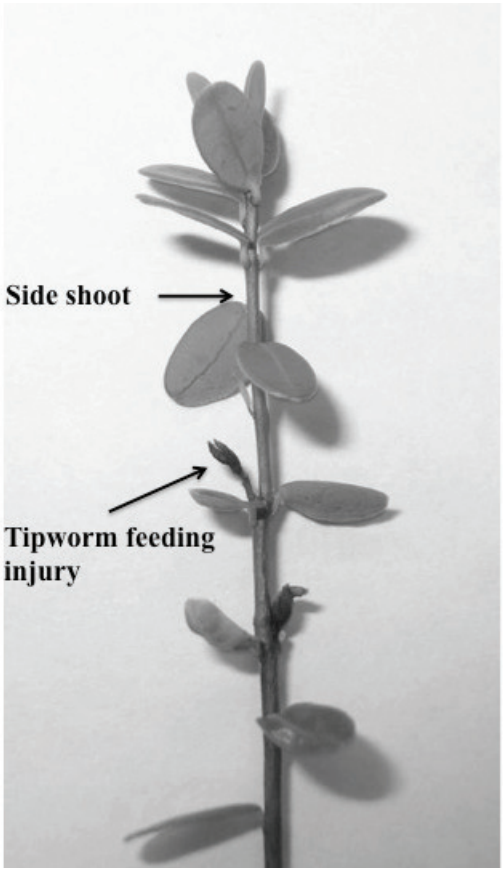

Figure 18. Vegetative cranberry upright with tipworm feeding injury and a side-shoot produced from lateral axillary bud.

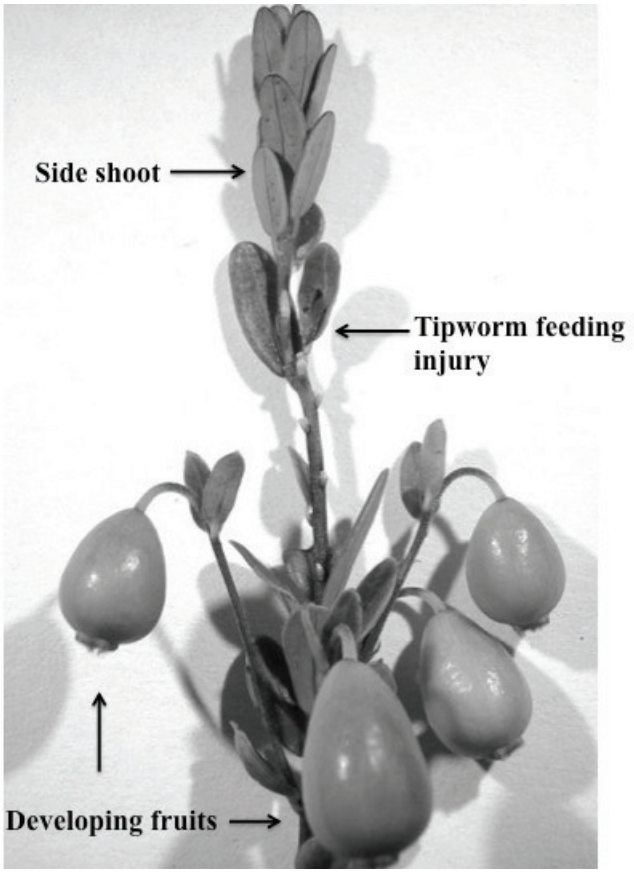

Figure 19. Flowering cranberry upright with tipworm feeding injury, side-shoot produced in response to the injury, and developing fruits in basipetal positions.

Materials and Methods

General methods (Field studies 1 and 2). The field work was carried out at a commercial cranberry farm with established beds of Howes and Stevens (41°56'59.31" N 70°47'15.98" W), planted in 1985 and 1995, respectively. For each cultivar, two 0.2 ha plots were established and four 10 m equidistant transects were marked (in each plot). The transects were laid down perpendicular to the direction in which the majority of runners was oriented to eliminate measuring more than one upright on a single runner.

Comparing flowering and vegetative uprights for the production of side-shoots after tipworm injury (Field Study 1)

One plot in each of the cultivar beds was used (Howes and Stevens). For each transect in both the cultivar plots, 15 flowering uprights and an equal number of vegetative uprights, all with visible tipworm feeding injury signs (cupping of terminal leaves with dead apical meristem), were identified and tagged in the first week of June 2011 using plastic slip-on tags printed with unique identification numbers (Texpak Inc., Franklin Square, NY). The two types of tagged tipworm-injured uprights were approximately evenly spaced along the entire length of each transect. A total of 60 flowering and 60 vegetative uprights were tagged for each cultivar (15 per transect × 4 transects). Data on the presence or absence of side-shoots were collected from all the tagged uprights in September 2011.

Determining impact of flower bud removal on the production of side-shoots in tipworm injured flowering uprights (Field Study 2)

One plot (separate from field study 1) in each of the cultivar beds was used. For each transect in both the cultivar plots, 45 flowering uprights with visible tipworm feeding injury signs (cupping of terminal leaves with dead apical meristem and characteristic scar tissue) were identified and tagged in the first week of June 2011 using plastic slip-on tags printed with unique identification numbers (Texpak Inc., Franklin Square, NY). The tagged uprights were approximately evenly spaced along the entire length of each transect. A total of 180 individual flowering uprights was tagged for each cultivar (45 per transect × 4 transects). None of the flower buds

were open at the time of tagging in any of the uprights. For each transect in both of the cultivar plots, the tagged uprights were assigned randomly to one of the following three treatments (15 per treatment): i) all the flower buds removed from the upright, ii) all the flower buds removed from the upright except one (at the base of 2011 season growth – the first formed flower bud of an upright), and iii) all the flower buds left intact. A razor blade was used to excise the flower buds. Both flower removal treatments were applied in the first week of June (2011). Data on presence or absence of side-shoots were collected from all the tagged uprights in September 2011.

Determining impact of flower bud removal on the production of side-shoots in tipworm injured flowering uprights (Greenhouse Study)

The greenhouse studies were carried out with Ben Lear, Howes, and Stevens. Uprights of Ben Lear with visible signs of tipworm feeding injury were collected from an established variety plot at the University of Massachusetts Cranberry Station (East Wareham, MA; 41°46'00.58" N 70°40'07.90" W) in the first week of June 2011. Similarly, tipworm-injured uprights of Howes and Stevens were collected from the commercial farm used in Field Study 1. In addition to current season growth, each harvested upright had ≈3 cm of previous season growth. For each cultivar, 40 uprights were planted individually in 10.2 cm square growing pots (Griffin Greenhouse and Nursery Supplies, Tewksbury, MA) filled with a 3:1 (sand: peat) mixture. On the following day, 20 uprights were selected randomly and all the flower buds were excised using a razor blade. The remaining 20 uprights with intact

flower buds served as controls. Cranberry vines are routinely propagated from field-harvested uprights and form roots readily upon planting. The plants were maintained under greenhouse conditions and once stigmas became exposed, all flowers were hand-pollinated (Sarracino and Vorsa 1991) using pollen collected from the same plots as the uprights. Data on the presence or absence of side-shoots were collected from all the plants in September 2011.

Data analysis

We used SAS v. 9.3 for all the analyses (SAS Institute 2011) and separate analyses were carried out for the different cultivars.

Comparing flowering and vegetative uprights for the production of side-shoots after tipworm injury (Field Study 1). For each of the four transects, the proportions (and standard errors) of flowering and vegetative uprights with side-shoots were estimated. These estimates were averaged over the four transects to determine the proportion and variance of tipworm-injured uprights with side-shoots (flowering and vegetative). The averaged estimates were used to compare production of side-shoots between tipworm-injured flowering and vegetative uprights by constructing an overall Wald χ^2 test of no difference (PROC IML) that accounts for the variance structure. This test allowed transects to be a random factor and also for the treatments being blocked on transects.

Determining impact of flower bud removal on the production of side-shoots in tipworm injured flowering uprights (*Field Study 2*). For each of the four transects, the proportions (and standard errors) of the uprights with side-shoots in the three

treatments were estimated. These estimates were averaged over the four transects to determine the proportion and variance for each of the three treatments and utilized to construct an overall Wald χ^2 test of no difference among the three treatments (PROC IML) that accounts for the variance structure. This test allowed transects to be a random factor and also for the treatments being blocked on transects. Pairwise comparisons among the three treatments were carried out using PROC IML.

Determining impact of flower bud removal on the production of side-shoots shoots in tipworm injured flowering uprights (Greenhouse Study). A chi-square based categorical data analysis test (PROC FREQ), with treatment as the explanatory variable, was used to determine if removal of flower buds had an impact on production of side-shoots in greenhouse planted uprights.

Results

Comparing flowering and vegetative uprights for the production of side-shoots after tipworm injury (Field Study 1)

A greater number of tipworm-injured vegetative uprights produced side-shoots, as compared to the injured flowering uprights for both the cultivars (Howes: $\chi^2 = 69.67$; df = 1; $P < 0.001$; Stevens: $\chi^2 = 235.87$; df = 1; $P < 0.001$; Fig. 20).

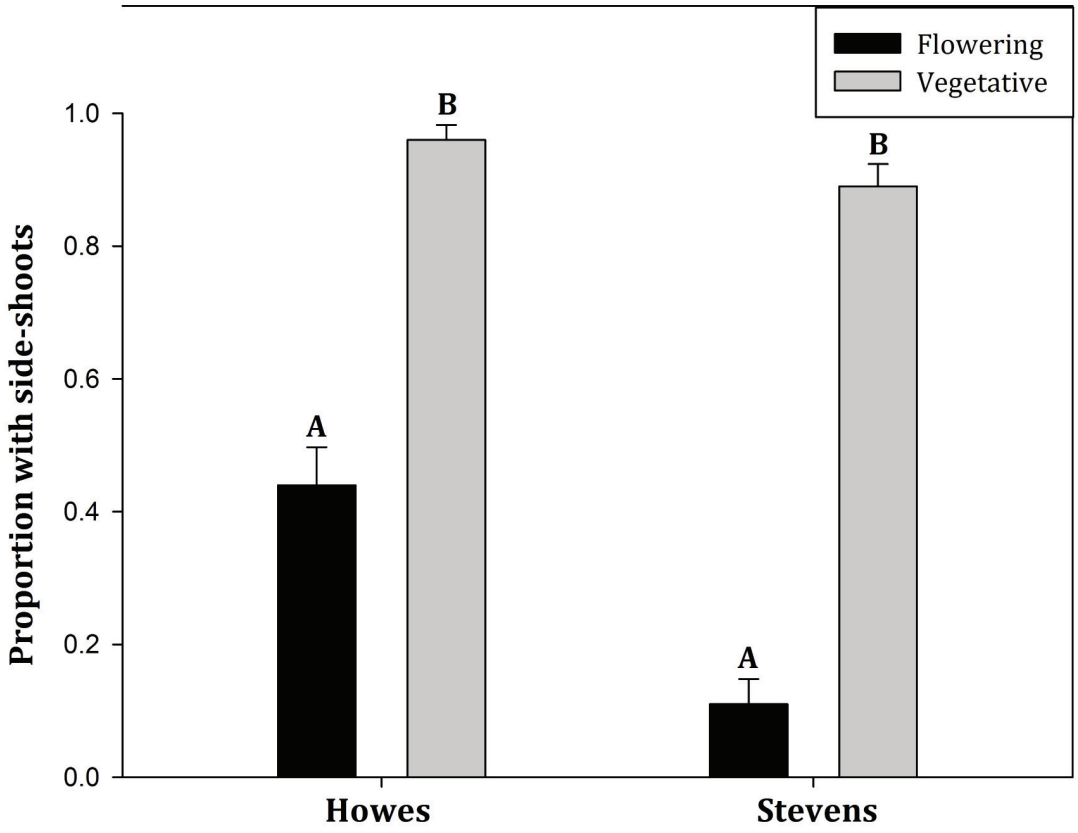

Figure 20. Estimated proportions of tipworm-injured flowering and vegetative uprights with side-shoots for Howes and Stevens. For each cultivar, significantly greater number of tipworm-injured vegetative uprights produced side-shoots as compared to the tipworm-injured flowering uprights ($\alpha = 0.05$).

Determining impact of flower bud removal on the production of side-shoots in tipworm injured flowering uprights (Field Study 2)

Both the flower bud removal treatments (T1 and T2) increased production of side-shoots in Howes and Stevens ($\chi^2 = 40.57$; df = 2; $P < 0.001$ and $\chi^2 = 38.83$; df = 2; $P < 0.001$; Fig. 21), as compared to the uprights with intact flower buds (C). In Stevens, a greater number of uprights from which all the flower buds were removed produced side-shoots, as compared to uprights in which one flower bud remained

(P = 0.002). There was no difference in side-shoot production between the two flower bud removal treatments for Howes (P > 0.05).

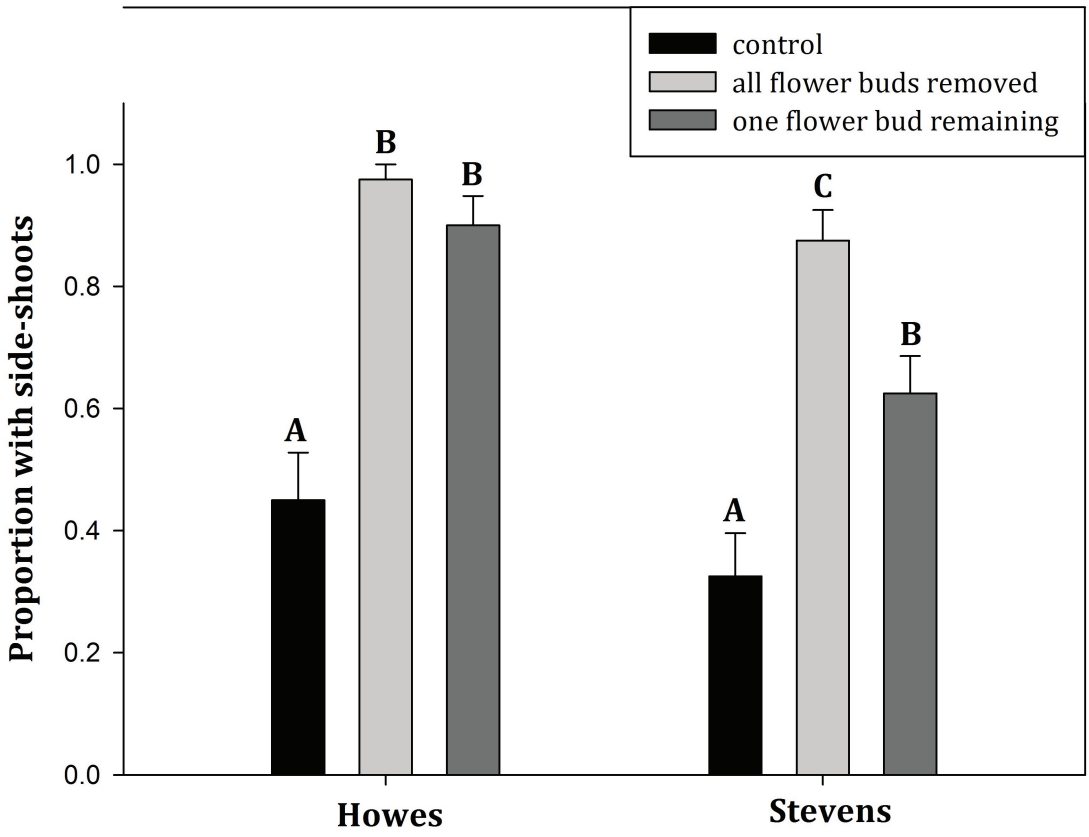

Figure 21. Estimated proportions of tipworm-injured flowering uprights with side-shoots for Howes and Stevens. The uprights either had all the flower buds intact (control), or subject to the two deblossoming treatments. For each cultivar, bars with the same letters above them are not significantly different (α = 0.05).

Determining impact of flower bud removal on the production of side-shoots in tipworm injured flowering uprights (Greenhouse Study)

Removal of flower buds from greenhouse planted uprights increased the production of side-shoots, as compared to the uprights with intact flower buds in Ben Lear,

Howes, and Stevens (χ^2 = 10.10; df = 1; P = 0.001; χ^2 = 5.71; df = 1; P = 0.01; χ^2 = 4.28; df = 1; P = 0.038; Fig. 22).

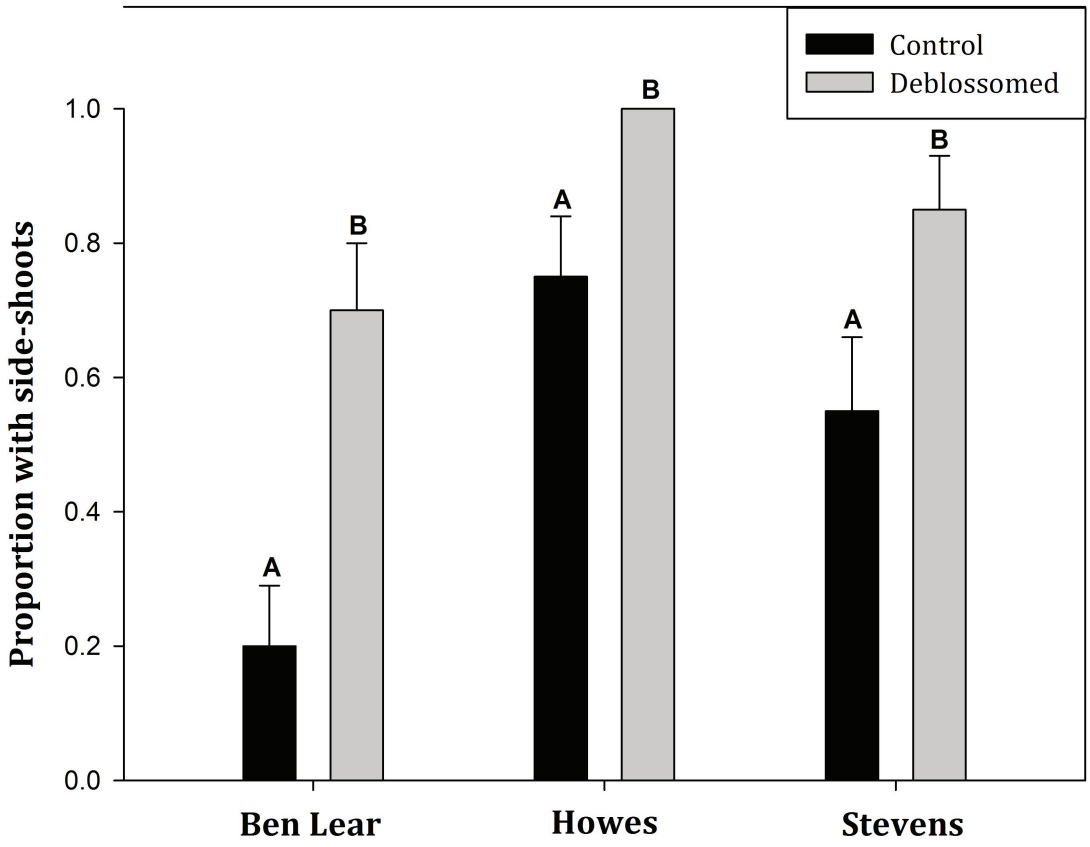

Figure 22. Estimated proportion of tipworm-injured flowering uprights with side-shoots for Ben Lear, Howes, and Stevens. The uprights either had all the flowers buds intact (control), or subject to the deblossoming treatment. For each cultivar, a greater number of deblossomed uprights produced side-shoots as compared to the uprights with flowers (α = 0.05).

Discussion

The key finding of our study was that presence of developing fruits inhibited the regrowth of tipworm-injured flowering uprights, and that removal of flower buds increased the production of side-shoots in all cultivars studied (Ben Lear,

Howes and Stevens). Furthermore, more vegetative uprights produced side-shoots as compared to flowering uprights after tipworm feeding injury. For Howes and Stevens, ≈ 40% and 20% of tipworm-injured flowering uprights resumed vegetative growth before the end of the growing season, respectively (Figs. 20). However, majority of tipworm-injured vegetative uprights (≈ 90%) of both the cultivars produced side-shoots before the onset of dormancy (Fig. 20). These results suggest that the partitioning of limited available resources between the potential sinks (developing fruits and dormant axillary buds) plays the key role in determining regrowth response of tipworm-injured cranberry uprights.

In Field Study 1, a greater number of tipworm-injured vegetative uprights produced side-shoots, as compared to the flowering uprights with developing fruits. The absence of additional sinks (developing fruits) in vegetative uprights may have resulted in activation of a greater number of dormant lateral buds, as compared to the flowering uprights. In the cranberry production region of Massachusetts, approximately two-thirds of all the uprights in any growing season are vegetative and results from our study demonstrate that a majority (≈90%) of the tipworm-injured vegetative uprights resume growth and produce side-shoots before the onset of dormancy. Tipworm injury is thus unlikely to have a significant impact on the growth and reproduction of vegetative uprights, from one growing season to next. However, the ability of a plant to compensate for herbivory may be affected by environmental factors such as length of the growing season (Huhta et al. 2000). Tipworm injury has also been reported from the cranberry production regions in the northern latitudes with a shorter growing season (Canada, Maine, and northern

Wisconsin) (C. Armstrong personal communication, Mahr and Perry 2006, Cook et al. 2011), and the regrowth responses of vegetative and flowering uprights are yet to be determined in these regions.

In cranberry, competition between vegetative and reproductive growth and limited availability of total nonstructural carbohydrates during fruit development has been reported (Birrenkott et al. 1991, Hagidimitriou and Roper 1994, Vanden Heuvel and DeMoranville 2009). Insufficient carbohydrate supply may result in competition between the different vegetative and reproductive sinks in plants and a number of studies have documented a negative relationship between fruit production and vegetative growth (Murneek 1926, Eaton 1931, Schaffer et al. 1986, Pakonen et al. 1988, Kappel 1991, Maust et al. 1999, Vaast et al. 2005). For example, deblossoming increased the shoot growth and leaf area of sweet cherry trees, *Prunus avium* L. (Kappel 1991). Similarly, Karlsson (1994) reported that the removal of flowers from the reproductive branches of *Rhododendron lapponicum* (L.) Wahlenb, an evergreen ericaceous clonal shrub, resulted in increased vegetative growth and hypothesized that resource competition was the underlying cause. Thus, competition for the limited available resources and a disproportionate partitioning of assimilates to the developing fruits over dormant axillary buds, may be responsible for the absence of regrowth in the majority of tipworm-injured flowering uprights.

In Field Study 2, removing either all the flower buds or all but one flower bud increased the number of uprights that produced side-shoots after tipworm feeding injury. Similar results were obtained in the greenhouse study wherein fewer

tipworm-injured uprights with intact flower buds produced side-shoots, as compared to deblossomed uprights in three different cultivars. Schaffer et al. (1985) reported that in strawberry (*Fragaria* × ananassa Duch.), a perennial clonal plant with aboveground runners, the accumulation of ^{14}C-assimilates in the youngest leaves declined with fruiting. However, developing leaves became the dominant sink for ^{14}C-assimilates after the removal of fruits. Similarly, Maust et al. (1999) reported that the number of vegetative buds that broke and grew in two cultivars of southern highbush blueberry (hybrids of *Vaccinium corymbosum* L.) decreased with increasing flower bud density. The partition of assimilates between two competing sinks depends on the intrinsic ability of each sink to receive assimilates relative to the other (Ho 1988). Results from our studies (Field Study 2 and Greenhouse) suggest that following apical meristem injury, developing fruits have a higher priority to receive the available assimilates as compared to the dormant axillary buds. Furthermore, priority of assimilate distribution can be altered through manipulation of a sinks' ability to draw in resources (Ho 1988), and may explain the higher rate of side-shoot production in the uprights that received the two flower bud removal treatments.

In Field Study 2, fewer uprights with one developing fruit produced side-shoots as compared to the uprights from which all the flower buds were removed in Stevens. However, there was no difference in side-shoot production between the two deblossoming treatments for Howes. Stevens produces relatively large sized fruits, as compared to the small-fruited Howes. It is therefore possible that the single developing fruit in Stevens sequestered a greater proportion of the available

assimilates, as compared to the Howes uprights with one fruit. Consequently, fewer uprights with one fruit may have initiated the growth of side-shoots in Stevens, as compared to the uprights from which all the flower buds were removed. However, phenological differences between the two cultivars may have also contributed to the different trends in production of side-shoots between the two flower bud removal treatments in Howes and Stevens.

In Field Studies 1 and 2, the majority of tipworm-injured flowering uprights with developing fruits failed to resume growth before the onset of dormancy (Howes and Stevens). Although injury to apical meristems (tipworm and artificial) does not impact the current season fruit production in cranberry (S. Tewari, unpublished data), uprights that fail to recover from tipworm feeding injury do not flower in the following year (Tewari et al. 2012). Tipworm-injured flowering uprights without side-shoots are also less likely to resume vegetative growth in the next growing season, as compared to injury free uprights (S. Tewari, unpublished data). The potential negative effects of upright-level lack of regrowth on commercial fruit production, from one growing season to next, have not been investigated in Massachusetts. The trend in data from the greenhouse study suggests a higher rate of side-shoot production for tipworm-injured uprights with intact flower buds (Howes and Stevens), as compared to the field tagged uprights of the same cultivars. Seventy-five and 55% of the greenhouse planted flowering uprights produced side-shoots as compared to 45 and 32% of the field tagged uprights for Howes and Stevens, respectively (Field Study 1). The change in growing habit of the upright, from clonal ramet (field) to physiologically independent and rooted plant

(greenhouse), may be responsible for the observed trend. Differences in irrigation and fertilization between the field and greenhouse may have also contributed to the above-mentioned trend.

In summary, our field and greenhouse studies demonstrate that presence of developing fruits inhibit regrowth of uprights after tipworm feeding injury. Furthermore, a greater number of vegetative uprights resumed growth as compared to the flowering uprights before the onset of dormancy. We now have a better understanding of the factors that affect the regrowth of cranberry uprights after tipworm feeding injury to the apical meristem. We suggest that competition among the different sinks and preferential allocation of the available assimilates to developing fruits inhibits the production of side-shoots in tipworm-injured flowering uprights.

CHAPTER 5

THE EFFECT OF INJURY TIME AND CULTIVAR ON THE TOLERANCE OF SIMULATED APICAL MERISTEM HERBIVORY IN CRANBERRY

Introduction

The impact of herbivory in plants depends on both the timing and the type of tissue attacked (Crawley 1997). Insect herbivory, such as galling of terminal buds, can result in the death of apical meristem tissue (DeClerck-Floate and Price 1994, Marquis 1996) and plants often respond by resuming growth from lateral axillary buds (Whitham and Mopper 1985, Marquis 1996, Haukioja and Koricheva 2000, Lehtila 2000, Wilson 2000, Bast and Reader 2003, Klimesova and Klimes 2007, Nakamura and Ohgushi 2007, Gruntman and Novoplansky 2011). The time of apical meristem injury is one of the factors that may impact regrowth and reproduction of perennial plants (Crawley 1983, Bilbrough and Richards 1993, Trumble et al. 1993, Watson 1995, Tolvanen and Laine 1997, Klimesova and Klimes 2007, Salemaa et al. 1999). The effect of injury time on regrowth may be modulated by physiological and morphological variables such as root-shoot ratios and proportion of photosynthetic surfaces in plants (Tiffin 2000).

The American cranberry (*Vaccinium macrocarpon* Aiton) is a woody, nondeciduous perennial and native to the North American continent (Eck 1990). Horizontal runners colonize the soil surface and produce vertical shoots, which are called uprights, at regular intervals. Each year the uprights may either produce vegetative growth only (vegetative uprights), or vegetative growth combined with multiple flowers (flowering uprights) (Fig. 23). Flowering uprights may put out 2 - 7

flowers at the beginning of the season, but approximately only half set fruit (Brown and McNeil 2006). Cranberry cultivars consist of selections from wild native stands (e.g., Howes), as well as those developed through traditional breeding programs (e.g., Crimson Queen and Stevens). Stevens was the result of a breeding program initiated by the USDA in 1929 to combat false blossom disease (Roper 2001). Crimson Queen was released recently from Rutgers University and is characterized by vigorous vine growth and high fruit yield.

Cranberry tipworm is a gall making fly whose larvae feed on the actively growing apical tissue of cranberry uprights and runners. After overwintering as late instar larvae, adults emerge around mid-May in Massachusetts and there can be multiple overlapping generations in a single growing season (Gagne 1989). Feeding injury by tipworm results in death of apical meristem tissue and vegetative growth of uprights is disrupted. However, uprights may resume growth in the current or subsequent growing seasons by producing side-shoots from lateral axillary buds (Voss 1996) (Fig. 23). It has been hypothesized that time of tipworm feeding injury (early vs. later in the growing season) plays a role in re-growth response of uprights (current season) and also affects flowering in the following year (Scammell 1917, Marucci 1960, Voss 1996, Mahr and Perry 2006). Specifically, it has been proposed that cranberry uprights are more tolerant of early season tipworm feeding as compared to injury sustained later in the growing season (Hardenberg 1908, Marucci 1960, Cockfield and Mahr 1994). In commercial cranberry farms of Massachusetts, peak tipworm feeding injury coincides with vegetative growth of uprights (early June – early July) and populations decline thereafter (S. T.,

unpublished data). Similar trends in population dynamics of tipworm have been reported from the cranberry production regions of Wisconsin (Mahr and Kachadoorian 1990, Cockfield and Mahr 1994, Voss 1996). After flowering, termination of vegetative growth eliminates the suitable oviposition sites for tipworm females (Cockfield and Mahr 1994).

We carried out a two-year field study (2010-2011) to determine the impact of early and mid-growing season apical meristem injury on reproductive and vegetative growth of three cranberry cultivars (Crimson Queen, Howes, and Stevens) in Massachusetts. Since the cultivars differ in phenology, we were also interested in determining if they varied in their response to the injury treatments (cultivar × injury interaction). Apical meristem excision of the uprights was used to substitute tipworm-feeding injury since there can be multiple overlapping generations of tipworm in a single growing season (Cockfield and Mahr 1994). Artificial injury has been used to simulate apical meristem herbivory previously (Bast and Reader 2003) and is an appropriate technique when the herbivore is not amenable to manipulation, or if precise treatments are required (Foggo 1996). The following questions were addressed in the present study: 1) Does time of apical meristem injury have an effect on vegetative regrowth, fruit set, and fruit biomass of uprights in the current growing season? 2) Does time of apical meristem injury impact growth and flower production of uprights in the next growing season?

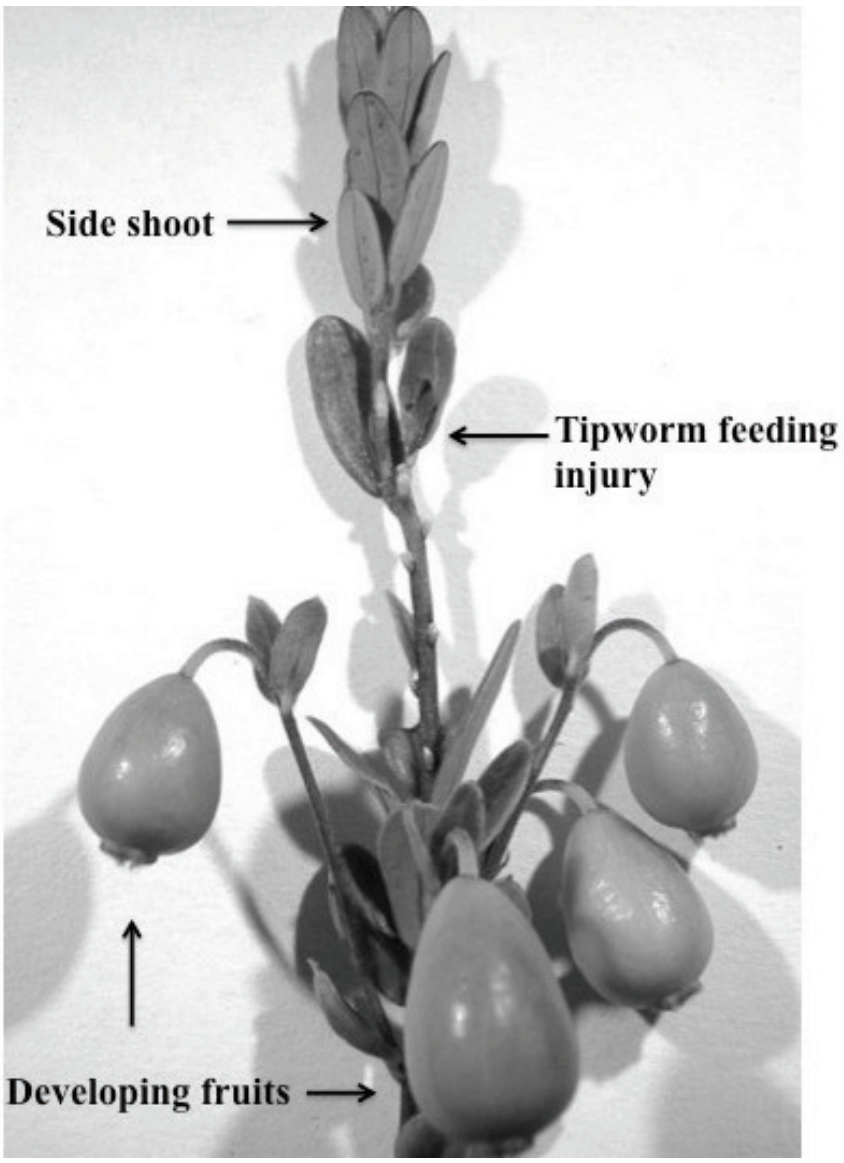

Figure 23. A flowering cranberry upright showing developing fruits in acropetal positions. Also visible is tipworm feeding injury to the apical meristem and a side-shoot produced from lateral axillary bud.

Materials and Methods

The study was carried out at the University of Massachusetts Cranberry Station (East Wareham, MA) (41°46'00.58" N 70°40'07.90" W). In 2007,

replicated plots of the three cultivars, each measuring 24 m × 12 m, were planted in a randomized pattern at the site. In 2010, for each of the three cultivars (Crimson Queen, Howes, and Stevens), three plots were chosen randomly for this study and in each plot three 12 m transects were marked equidistant from one another. The transects were laid down perpendicular to the direction in which the majority of runners was oriented to eliminate tagging more than one upright on a single runner. In each transect, 51 individual flowering uprights were tagged (June 2010) using plastic slip-on tags (Texpak Inc., Franklin Square, NY) printed with a number series. The tagged uprights were evenly distributed along the length of each transect. The following three treatments were applied randomly to an equal number of the tagged uprights (17 per treatment) in each transect: i) apical meristem excised using razor blade in the first week of June 2010 (early-season damage), ii) apical meristem excised in the first week of July 2010 (mid-season damage), and iii) apical meristem intact (control). Thus, for each cultivar, a total of 459 individual flowering uprights were tagged (153 per plot × 3 plots). Data on the presence or absence of side-shoots were collected from the tagged uprights in September 2011. Fruit set in the tagged uprights was determined at the beginning of September 2010 by dividing the number of fruits per upright by the number of flowers present at the beginning of study. Under-developed fruits or pin-heads (diameter < 0.5 cm) were not included in the calculation of fruit set. An outbreak of fruit scald at the study site late in the growing season prevented us from comparing the fruit weight among the treatments and cultivars in 2010.

The following year (June 2011), we collected data on the presence or absence of new growth and flowers from the tagged uprights. Thirty-seven, 43, and 47 uprights tagged in 2010 could not be located in 2011 for the control, early-season damage, and mid-season damage treatments, respectively. The missing uprights were approximately evenly distributed among the three cultivars.

Data Analysis

We used SAS v. 9.3 for all the analyses (SAS Institute 2011). Analysis of variance (ANOVA) (PROC MIXED) with an unstructured variance – covariance matrix was used to determine the impact of injury time (early-season vs. mid-season), cultivar, and their interaction on vegetative regrowth and fruit set (2010). We also carried out separate analyses comparing the three cultivars for the two injury treatments. Similar analyses were used to determine the impact on vegetative growth and flower production in the next growing season (2011). Transect was treated as a random effect (nested within plots) with the observations on the different treatments as repeated measure on the transect. We did not assume independence among the observations within a transect because of possible spatial effects. Plot was included as a fixed factor throughout the analysis. Analysis was carried out on the means of data from the 17 uprights of each of the three treatments per transect. In the case of vegetative regrowth (2010), vegetative growth (2011), and flower production (2011), the means are proportions.

Results

Vegetative regrowth – side-shoots (2010)

Overall, fewer flowering uprights subject to early-season simulated tipworm feeding injury produced side-shoots, as compared to uprights injured in mid growing season ($F = 44.24$; $df = 1, 6$; $P = 0.0006$). There was a significant cultivar effect ($F = 24.08$; $df = 2, 5$; $P = 0.0027$) and a higher number of Howes uprights produced side-shoots, as compared to uprights of Crimson Queen and Stevens ($P = 0.0026$; $P = 0.0015$; both Tukey adjusted). There was no difference between Crimson Queen and Stevens in production of side-shoots ($P > 0.05$). There was a significant treatment × cultivar interaction for the production of side-shoots ($F = 11.23$; $df = 2, 5$; $P = 0.0142$). On comparing the two injury treatments by cultivar, a significantly higher number of uprights with mid-season injury produced side-shoots for Howes, as compared to uprights with early-season simulated tipworm injury ($F = 37.33$; $df = 1, 6$; $P = 0.0009$; Fig. 24). For Crimson Queen and Stevens a numerically higher number of uprights with mid-season injury produced side-shoots, as compared to uprights with early-season simulated tipworm injury (Fig. 24). On comparing the three cultivars for each injury treatment, a greater number of Howes uprights produced side-shoots in response to both early and mid-season injury, as compared to uprights of Crimson Queen (early – $P = 0.0152$; mid – $P = 0.0013$; both Tukey adjusted; Fig. 24) and Stevens (early – $P = 0.0111$; mid – $P = 0.0021$; both Tukey adjusted; Fig. 24). There was no difference between Crimson Queen and Stevens for side-shoot production (both early and mid-season injury; Fig. 24).

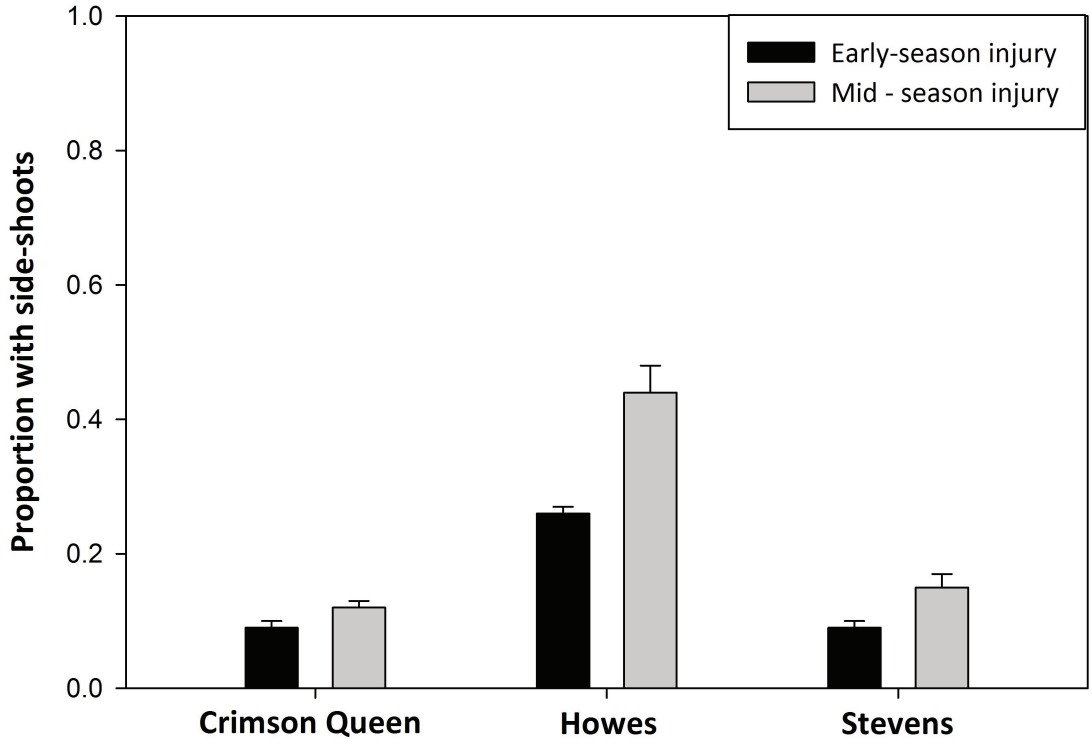

Figure 24. The proportion of uprights that produced side-shoots (mean ± SE), after early and mid-season simulated tipworm injury in three cranberry cultivars (Crimson Queen, Howes, and Stevens). There was significant difference in the production of side-shoots between the two treatments for Howes, but not for Crimson Queen and Stevens.

Fruit set (2010)

Early and mid-growing season simulated tipworm injury did not have an impact on fruit set compared to the control ($P > 0.05$). Cultivars differed significantly in fruit set ($F = 57.38$; $df = 2, 18$; $P < 0.0001$), which was 0.45 (±0.01), 0.54 (±0.01), and 0.32 (±0.01) for Crimson Queen, Howes, and Stevens, respectively. Furthermore, there was no treatment × cultivar interaction ($P > 0.05$) for fruit set.

Vegetative growth of uprights (2011)

There was a significant treatment effect ($F = 55.97$; $df = 2, 17$; $P < 0.0001$) and fewer uprights with either early or mid-growing season injury (2010) resumed growth in 2011, compared to the intact control uprights ($P < 0.0001$ – both comparisons; both Tukey adjusted). This result was consistent for all three cultivars (Fig. 25). There was no difference in vegetative growth between the uprights that received the two injury treatments ($P > 0.05$). There was a significant cultivar effect ($F = 40.71$; $df = 2, 18$; $P < 0.0001$) and greater number of Howes uprights produced new growth in 2011 as compared to Crimson Queen and Stevens ($P < 0.0001$; $P = 0.0011$; both Tukey adjusted). Furthermore, greater number of Stevens uprights produced new growth as compared to uprights of Crimson Queen ($P = 0.0005$; Tukey adjusted). There was no treatment × cultivar interaction for vegetative growth in 2011. On comparing the three cultivars for each injury treatment, a greater number of Howes uprights with early and mid-season injury resumed growth, as compared to uprights of Crimson Queen that received the two injury treatments (early – $P < 0.0001$; mid – $P = 0.0013$; both Tukey adjusted). In addition, a greater number of Howes uprights with early season injury resumed growth, as compared to Stevens uprights with early season injury ($P = 0.0441$; Tukey adjusted).

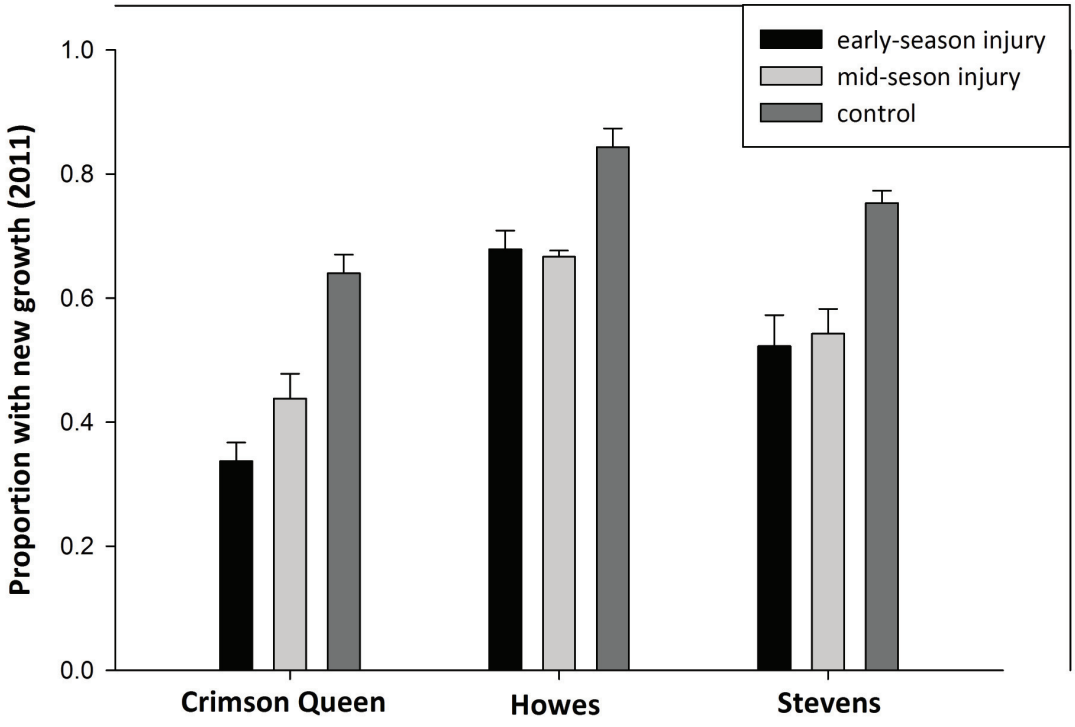

Figure 25. The proportion of uprights that produced new growth (mean ± SE) in 2011, after early and mid-season simulated tipworm injury in the previous growing season.

Flowering (2011)

There was a significant treatment effect ($F = 16.11$; $df = 2, 17$; $P = 0.0001$) and fewer uprights with either early or mid-growing season injury (2010) produced flowers in 2011, compared to the intact control uprights (early – $P < 0.0001$; mid – 0.0003; both Tukey adjusted). There was no difference between the uprights that received the two injury treatments for flower production ($P > 0.05$). There was a significant cultivar effect ($F = 11.36$; $df = 2, 18$; $P = 0.0006$) and fewer uprights of Crimson Queen produced flowers as compared to Howes and Stevens ($P = 0.0007$; P

= 0.0068, both Tukey adjusted). There was no difference between Howes and Stevens in production of flowers (P > 0.05). There was no treatment × cultivar interaction for flower production (P > 0.05). On comparing the three cultivars for each injury treatment, a greater number of Howes uprights with early-season injury produced flowers, as compared to uprights of Crimson Queen (P = 0.0037; Tukey adjusted) and Stevens (P = 0.0358; Tukey adjusted) that received the same treatment (Fig. 26).

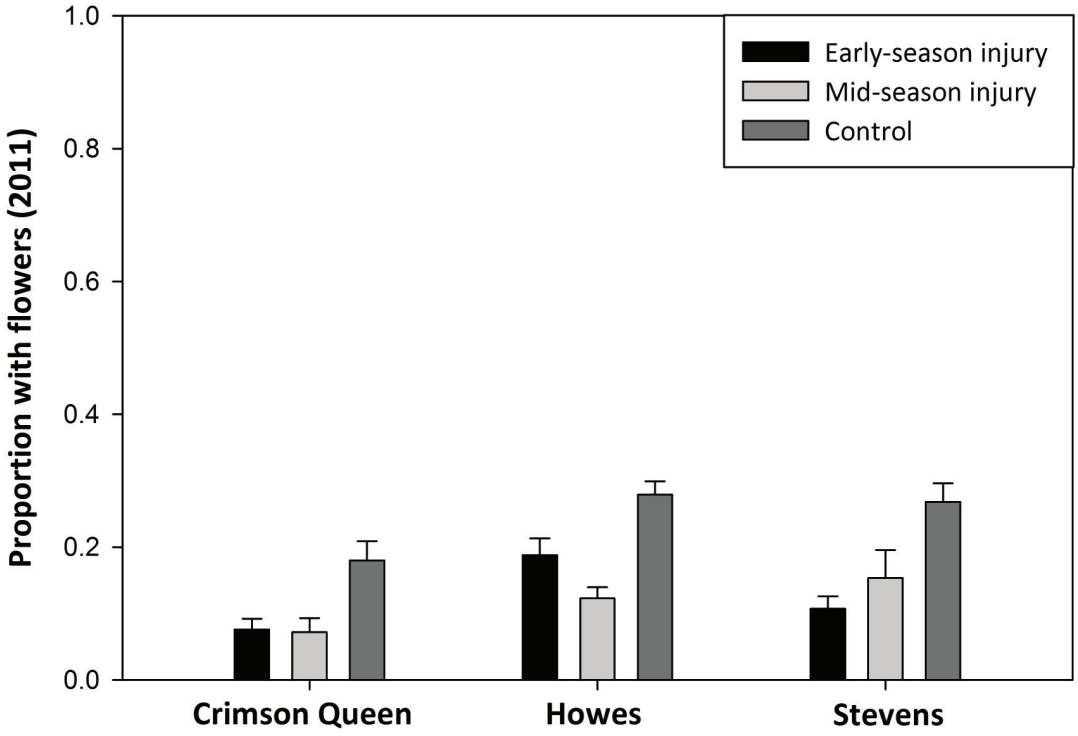

Figure 26. The proportion of uprights that produced flowers (mean ± SE) in 2011, after early and mid-season simulated tipworm injury in the previous growing season.

Discussion

The key finding of our study was that both early and mid-season simulated tipworm herbivory inhibited the growth and flowering of cranberry uprights in the following year. The result was consistent among the three cultivars and contrasts with earlier reports, which suggested that uprights injured relatively early in the season can recover and resume normal growth in the following year (Hardenberg 1908, Marucci 1960, Cockfield and Mahr 1994). It has still not been determined if apical meristem injury (tipworm or artificial) can impact the survival of cranberry uprights from one growing season to next. The inhibition in growth of injured uprights documented in our study (2011) may thus be either temporary, or the result of upright mortality. Notwithstanding, the long-term effects of injury may be significant since vegetative growth plays an important role in the life history of perennial clonal plants (Stevens et al. 2008). Cranberry uprights accrue annual vegetative growth and eventually become decumbent (Eck 1990). Inhibited growth of injured uprights may therefore impact the clonal architecture of cranberry since the lateral buds of decumbent uprights can produce more uprights.

After tipworm injury or apical meristem excision, cranberry uprights can resume growth by producing side-shoots from lateral axillary buds (Fig. 23). A greater number of uprights injured later in the growing season produced side-shoots in Howes, as compared to uprights with early season injury. The trend was similar for Crimson Queen and Stevens. The result suggests that uprights are more tolerant when apical meristem injury occurs at a phenologically advanced stage of growth. This phenomenon in turn may be governed by factors such as temporal

variation in the resource allocation patterns, or in the sensitivity of lateral axillary buds to resume growth after injury.

Howes exhibited greater tolerance of apical meristem injury and more uprights produced side-shoots in the current growing season, as compared to injured uprights of Crimson Queen and Stevens (both injury treatments). Difference between the native (e.g., Howes) and hybrid (e.g., Crimson Queen and Stevens) cultivars in per unit area fruit productivity may be responsible for the pattern of regrowth documented in our study. For example, Stevens plantings on average produce ≈50% more fruit by weight than Howes in Massachusetts (DeMoranville 2011). In cranberry, an inverse relationship may exist between investment in reproductive output and the ability to regrow after apical meristem injury.

Differences in the production of side-shoots among the three cultivars may have also affected growth and flowering in the following year. In 2011, a greater number of Howes uprights subjected to early and mid-season injury resumed growth than the uprights of Crimson Queen and Stevens (except when compared to Stevens uprights with mid-season injury). Similarly, a greater number of Howes uprights with early season injury produced flowers in the following year, as compared to Crimson Queen and Stevens. Although our study demonstrates higher tolerance of apical meristem injury in Howes, the results should be interpreted with caution. Comparison among commercial cranberry production sites in Massachusetts documented greater proportions of uprights with tipworm injury at Howes plantings, as compared to those with Stevens (Tewari et al. 2012). Thus, any

potential benefits of enhanced tolerance in Howes may be offset by a higher overall tipworm injury rate (relative to the other cultivars).

Fruit set in the current growing season (2010) was not affected by either of the simulated injury treatments. Scald symptoms at the end of the growing season prevented us from comparing fruit weight among the treatments. However, fruit set is a key component of berry yield at the upright level (Eaton et al. 1983) and we may extrapolate the result of no difference among treatments to fruit output. Tipworm larvae feed and complete development on actively growing uprights (Cockfield and Mahr 1994, Voss 1996) and oviposition declines sharply after flowering (early July). The two injury treatments in our study covered the range of peak tipworm activity in Massachusetts (early June – early July) and results demonstrate that current season fruit output is unaffected by apical meristem injury to the uprights. In addition, site and plot level studies in Massachusetts suggest that tipworm injury does not result in yield loss from one growing season to next (Tewari et al. 2012). However, tipworm is considered a pest in the cranberry growing regions of Maine, Canada, and northern Wisconsin (C. Armstrong personal communication, Cook et al. 2011, Mahr and Perry 2006). Tewari et al. (2012) reported a trend wherein commercial cranberry production sites in Maine had a greater proportion of uprights with tipworm injury, as compared to sites in Massachusetts. The effects of both early and mid-season tipworm suppression must be investigated in these regions to determine if injury can impact fruit yield in the following year.

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